STUDIES ON THE RELATIONSHIPS BETWEEN INCIDENCE OF UROLITHIASIS AND MINERAL METABOLISM IN FATTENING CATTLE

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1976

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CONTENTS

INTRODUCTION ---------- 1

CHAPTER 1. INCIDENCE OF URINARY CALCULI AND ITS EFFECT ON URINARY ORGANS IN FATTENING STEERS ---------- 3

Materials and Methods ---------- 3

Results ---------- 4

Discussion ---------- 9

Summary ---------- 14

CHAPTER 2. CHEMICAL COMPOSITION OF URINARY CALCULI IN FATTENING CATTLE ---------- 15

Materials and Methods ---------- 15

Results ---------- 17

Discussion ---------- 20

Summary ---------- 25

CHAPTER 3. RELATIONSHIP BETWEEN URINE AND SERUM MINERAL LEVELS AND INCIDENCE OF URINARY CALCULI IN FATTENING STEERS ---------- 27

Materials and Methods ---------- 27

Results ---------- 29

Discussion ---------- 34

Summary ---------- 38

CHAPTER 4. EFFECT OF FEEDING HIGH CONCENTRATE RATIONS ON MINERAL METABOLISM AND INCIDENCE OF URINARY CALCULI ---------- 39

SECTION 1. Effect of Feeding High Concentrate Rations on Urine and Serum Mineral Levels in Wethers ---------- 39
Materials and Methods ---------- 39
Results ---------- 40
Discussion ---------- 49
Summary ---------- 53
SECTION 2. Relationship Between Urine Mineral Levels and Rumen Fermantation in Wethers Fed with High Concentrate Ration ---------- 54
Materials and Methods ---------- 54
Results ---------- 57
Discussion ---------- 65
Summary ---------- 68
CHAPTER 5. EFFECT OF DIETARY CALCIUM AND PHOSPHORUS LEVELS ON MINERAL METABOLISM AND INCIDENCE OF URINARY CALCULI ---------- 69
SECTION 1. Effect of Addition of Calcium Carbonate on Mineral Metabolism and Incidence of Urinary Calculi in Wethers ---------- 69
Materials and Methods ---------- 70
Results ---------- 73
Discussion ---------- 78
Summary ---------- 83

SECTION 2. Effect of Dietary Calcium on Mineral Level and Its Solubility of Digesta in Wethers -------- 84
Materials and Methods ---------- 84
Results ---------- 87
Discussion ---------- 95
Summary ---------- 99
CHAPTER 6. EFFECT OF VITAMIN A DEFICIENCY ON MINERAL METABOLISM AND INCIDENCE OF URINARY CALCULI ---------- 101
Materials and Methods ---------- 101
Results ---------- 104
Discussion ---------- 109
Summary ---------- 114
CHAPTER 7. EFFECT OF ADDITION OF AMMONIUM CHLORIDE ON MINERAL METABOLISM AND INCIDENCE OF URINARY CALCULI ---- 115
SECTION 1. Effect of Ammonium Chloride Administration on Urine and Serum Mineral Levels in Wethers --- 115
Materials and Methods ---------- 116
Results ---------- 118
Discussion ---------- 122
Summary ---------- 126
SECTION 2. The Mechanism of Ammonium Chloride on the Prevention of Urolithiasis ---------- 127
Materials and Methods ---------- 127
Results ---------- 129
Discussion ---------- 133

Summary ---------- 137

GENERAL DISCUSSION AND CONCLUSION ---------- 139

ACKNOWLEDGEMENT ---------- 146

REFERENCES ---------- 147

INTRODUCTION

Urolithiasis is characterized by the formation of calculi in the kidney, bladder and urethra with resultant obstruction of urine excretion.

The first symptoms of urolithiasis are an white opaque appearance in urine and white precipitates at the prepuital hair. Then, animals show dysuria, anorexia and painful manners. When the worst symptom of urolithiasis appears, the animal must be slaughtered immediately. The words, urolithiasis, was used in this study when a harmful effect was given to animals with this disease.

The chemical composition of urinary calculi was investigated in Australia[59], New Zealand[2] and USA[5,7]. Blout[7] lists seven types of calculi in cattle, namely, silicate, calcium oxalate, xanthine, calcium and magnesium carbonates, calcium and magnesium phosphates, triple phosphate and iron carbonate.

Although the chemical composition of urinary calculi was well established, the causative factor of urolithiasis is still remarkably confused.

It has been known in Japan that the urolithiasis was induced in poultry and sheep, while these urinary calculi were mainly composed of uric acid and calcium respectively[103]. In fattening cattle, the incidence of urolithiasis appeared to increase from about 1965.

Recently the occurrence of urolithiasis has been frequently observed among fattening cattle in Japan and caused considerable

damage to livestock production. For example, the percentage of urolithiasis in fattening cattle slaughtered because of the disease was 7 % in Japan (1973)[47] and was 6.5 % in Kyoto prefecture (1974)[44].

A feedlot operation, in which high concentrate rations were given in order to shorten the fattening period, has prevailed in Japan from about 1965 and the occurrence of urolithiasis in fattening cattle seems to have increased from that time.

The author began to study the association between the etiology of urolithiasis and dietary factors. In this process it was found that the formation of urinary calculi was closely associated with mineral metabolism. The purpose of this study is to clarify the relationships between the incidence of urolithiasis and mineral metabolism.

CHAPTER 1 INCIDENCE OF URINARY CALCULI AND ITS EFFECT ON URINARY ORGANS IN FATTENING STEERS

Anatomical and histological studies on urinary organs in sheep suffered from urolithiasis have been conducted by Pontius et al.[69] and Cornelius and Moulton[13]. However, these workers used sheep and only studied the morbid changes at autopsy.

As a preliminary step to determine the etiology of urolithiasis, this experiment was conducted to find where urinary calculi lodged and to determine the relationship between calculi formation and the morbid changes of urinary organs in fattening steers.

Materials and Methods

This experiment, using two Wagyu steers, was conducted for 240 days, beginning in summer and ending in the next spring. These steers full-fed the concentrate ration containing 70 %, steam-rolled milo; 10 %, wheat bran; 10 %, rice bran; 8 %, soybean meal; 1 %, sodium chloride; 1 %, calcium carbonate; and the roughage, mainly Italian ryegrass.

One steer (A steer) showed normal growth and its average daily gain was over 1.0 kg/day. However, this animal showed anorexia, dysuria and painful manners, and later the inability to urinate at the end of the experiment. The body weight of the

other stter (B steer) hardly increased after three months from the beginning of this experiment, and the symptoms of urolithiasis also appeared and the urination of the animal stopped at the end of this experiment.

When the symptoms of urolithiasis got worse, 30 g of ammonium chloride was given to each steers daily for two weeks before slaughter, but it did not treat urolithiasis, and urination was completely stopped for a few days. Therefore, both animals were slaughtered, and the post mortem examination was conducted.

The kidney, the bladder and the urethra were examined to find the incidence of urinary calculi and lesions. Sample tissues were collected from these viscera in order to conduct microscopic examination. Each of tissue samples was placed in formalin, and then studied in two ways, with hematoxylin and eosin and with hematoxylin and PAS.

Results

Hemorrhage and infiltration were found around the kidney of A steer. Kidneys were slightly enlarged. The weight of right kidney was 450 g and left one was 470 g. The color of right kidney was normal, while that of left kidney was partially decolorized. The separation of capsule fibrosa was difficult at both kidneys.

In B steer, hemorrhage and infiltration in the fat around the kidneys were less than those of A steer. However, kidneys were enlarged and were two or three times heavier than those of normal weight. The weight of right kidney was 970 g and left kidney was 1250 g. As shown in fig. 1-1, the color of kidneys were severely decolorized all over the surface. The separation of capsule fibrosa was difficult for both kidneys.

Many calculi were found in most of the renal calix of both steers (fig. 1-2). Renal calculi found in A steer were large and hard concretions, and showed whitish color. While the calculi found in B steer were sand-like stones. The result of histopathological study of the kidneys is shown in table 1-1.

Microscopic pictures of samples from A steer showed the disappearance of glomerulus (fig. 1-5), compensatory enlargement of glomerulus, hyaline degeneration in glomerulus, thickening glomerulus basement membrane (fig. 1-6) and morbid changes of renal tubule. These observation suggested that the kidneys from A steer had suffered with chronic nephritis.

In B steer, glomerular changes were comparatively less. However, renal tubule and interstitial tissue were remarkably degenerated. Round cells infiltration into interstitial tissue and edema in epithelium cells and interstitial tissue (fig. 1-7) appeared to show chronic inflamation of the kidney. Suppurative foci (fig. 1-8) were also observed in some parts. Interstitial connective tissue appeared to be progressively increased and

Table1-1 Characteristics of morbid changes of the kidney by microscopic examination

	Steer	
	A	B
Glomerulus		
Disappearance	+	+
Disappearance of nuclei	++	+
Compensatory enlargement	++	
Hyaline degeneration	+	
Fattening of basement membrane	+	
Renal tubule		
Dilatation	++	++
Protein urinary casts	++	
Disappearance of brush border	+++	
Atrophy and disappearance		+++
Vacuolor degeneration of epithelium	++	+
Interstitial tissue		
Round cells infiltration		+
Suppurative foci		+
Increasing in number of cells		++
Edema		++

The symbols, +, ++, +++, show the severity of lesions (+: mild, ++: moderate, and +++: severe).

many renal tubuli appeared to be involved and disappeared (fig. 1-8). These changes suggested that there was serious renal insufficiency. The function of the kidney of B steer appeared to be more disturbed than that of A steer.

The tissue of the bladder appeared to become thin and severe hemorrhage was found in the mucosa of the bladder (fig. 1-3). The hemorrhage found in B steer's bladder was more severer than that of A steer. Many globular and glassy calculi like rice were found in the bladders of both steers. These calculi were quite hard and were not crushed by fingers, and were different from those of renal calculi.

It was observed that urinary calculi, about 0.6 cm in diameter, filled the end of S-shaped curve of the urethra of both steers (fig. 1-4). From the bladder to the end of S-shaped curve of the inner urethra, severe hemorrhage was found, but the urethra beyond the S-shaped curve was not affected at all. These grayish white calculi consist of small and sand-like calculi. The result of histopathological study of the bladder and the urethra is shown in table 1-2.

Hemorrhage, edema and necrosis found in the mucosa and the muscle of the bladder of both steers indicate cystitis in the bladder. The intensity of cystitis appeared to be more severe in B steer than in A steer, because water lodged cells (fig. 1-9), round cells infiltration and suppurative foci were found in the bladder of B steer. Severe hemorrhage and round cells infiltration

Table 1-2 Characteristics of morbid changes of the bladder and the urethra by the microscopic examination

	Steer	
	A	B
Bladder		
Hemorrhage in mucosa and muscle	++	++
Edema in mucosa and muscle	++	++
Necrosis of muscle	+	+
Increase in number of transitional epithelium cells		++
Water lodged cells in transitional epithelium		+
Round cells infiltration in mucosa		+
Suppurative foci in mucosa		+
Urethra		
Hemorrhage and congestion	++	+++
Desquamation of epithelium	++	++
Round cells infiltration		+
Formation of glanulation tissue		+

The symbols, +, ++, +++, show the severity of lesions (+: mild, ++: moderate, and +++: severe).

found in the inner tissue of the urethra indicated that the urethra suffered from urethritis. The formation of granulation tissue was found in the urethra of B steer (fig. 1-10).

Discussion

When urinary calculi were present at the prepuce, both steers suffered from dysuria. When they were removed from the prepuce, dysuria disappeared. Therefore, calculi at the prepuce may be one of the causes of dysuria in fattening steers. Since urinary calculi were occasionary found in the bladders of normal animals, the presence of calculi in the bladder may not be the main causes of urolithiasis. However, the urinary calculi was found at the S-shaped curve of the urethra in both steers having urolithiasis, and it was considered that inability to urinate may be caused by the presence in the urethra. Pontius et al.[69] and Newsom et al.[61] had previously reported that urinary calculi in the urethra would be a direct cause of urolithiasis in sheep.

The considerable morbid changes were observed in the kidneys of both steers at autopsy and by the microscopic examination. Several workers[57,61,69] observed the dilatation of the kidneys in sheep suffered from urolithiasis. However, Beeson et al.[5] reported that the very little damage was found in the kidney structure of sheep having urolithiasis. The renal organs of B steer showed greater changes than the other steer. This

animal had been frequently suffered from dysuria since relatively earlier period of fattening.

There may be the positive relationship between the duration of dysuria and the degree of morbid changes in the urinary organ. If an animal is slaughtered shortly after finding the symptoms of urolithiasis, the kidney may not be damaged much because of the short period of dysuria. Most of renal morbid changes may not be the primary cause of calculi formation in steers, because urolithiasis was found even in animals not having renal morbid changes. Desquamated epithelium of the kidney may form the nuclei of urianry calculi. Further studies would be necessary to find the effect of renal morbid changes on the formation of urianry calculi.

Fig. 1-3 Enlargement and decolorized kidneys of B steer.

Fig. 1-4 Many renal calculi were found in almost every renal calix.

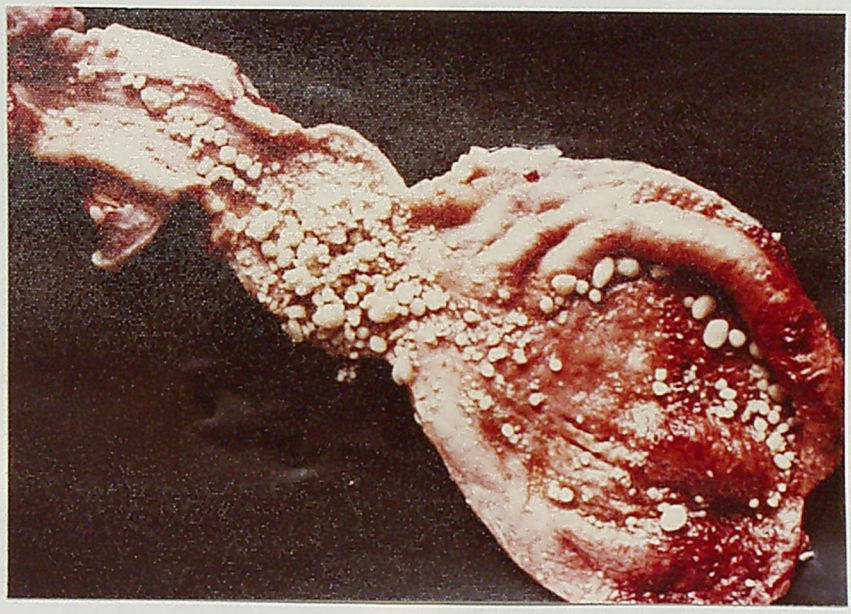


Fig. 1-5 Severe hemorrhage in the mucosa of bladder and small globular urinary calculi.

Fig. 1-6 Lodging of a urinary calculus at the end of S-shaped curve of urethra.

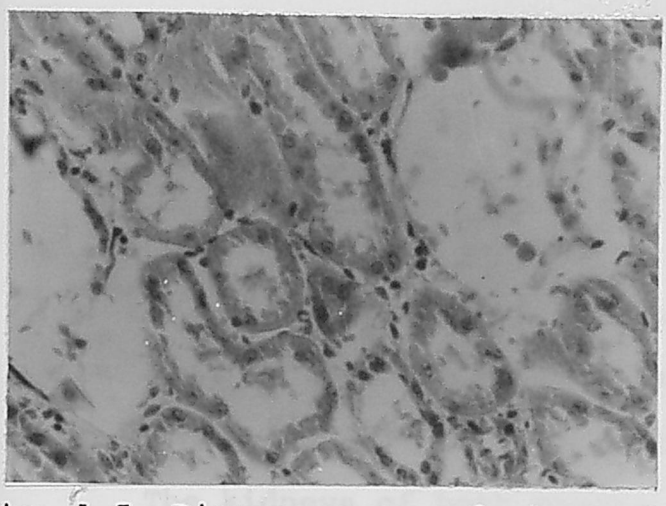


Fig. 1-5 Disappearance of glomerulus and protein substances, x 140, HE staining.

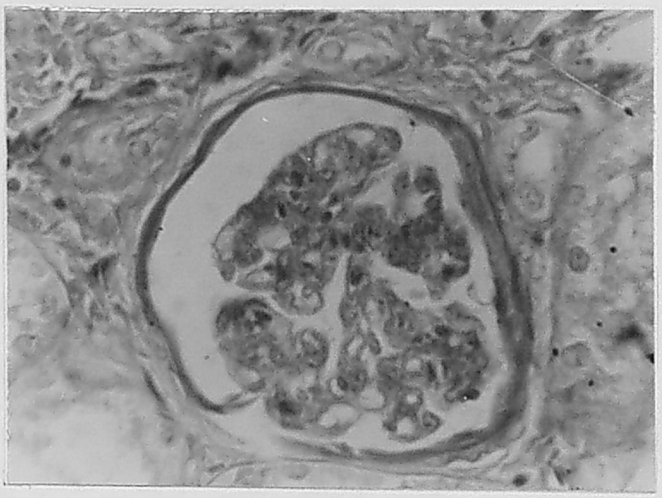


Fig. 1-6 Enlargement of glomerular basement membrane, x 280, HP staining.

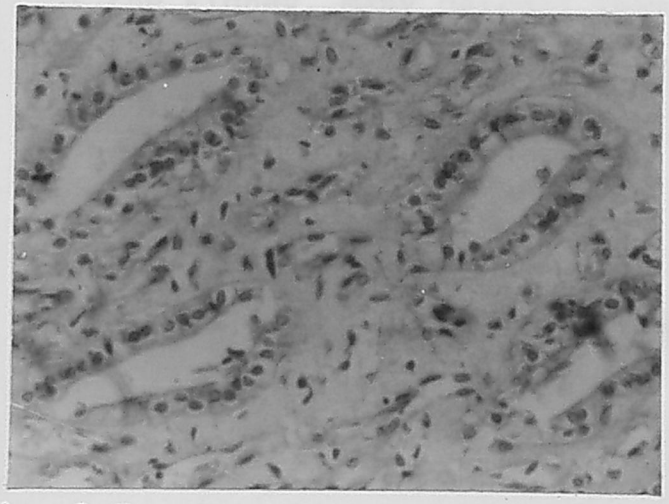


Fig. 1-7 Edema in renal epithelium cells and interstitial tissue, x 150, HE staining.

Fig. 1-8 Suppurative foci, increasing in number of connective tissue cells, and involuting and disappearing of renal tubule, x 140, HE staining.

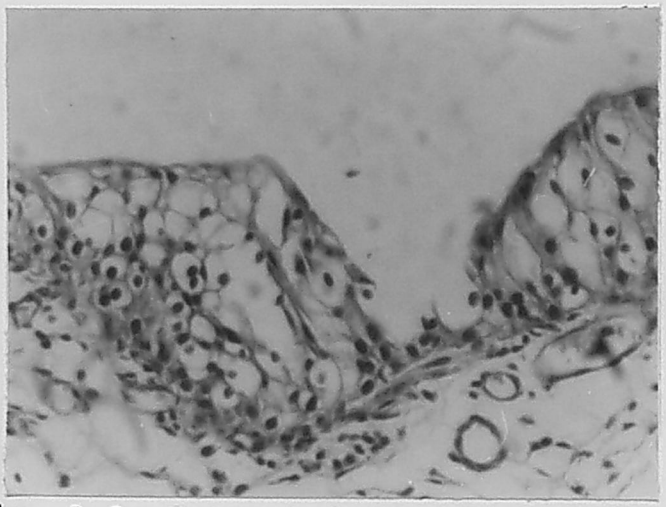


Fig. 1-9 Water lodged cells in interstitial epithelium of bladder, x 150, HE staining.

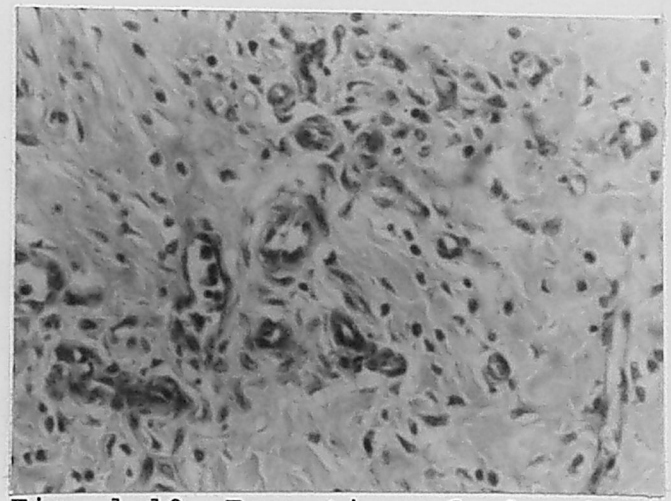


Fig. 1-10 Formation of glanulation tissue in urethra, x 150, HE staining.

Summary

Two fattening steers having urolithiasis were slaughtered to find where urinary calculi had lodged and to observe the morbid changes of the urinary organs.

The kidneys of both steers were slightly enlarged and decolorized. It was difficult to separate the capsule fibrosa of the kidney. Many calculi were found in most of the renal calix. By histopathological study of the kidneys, chronic nephritis was observed in one steer and serious renal insufficiency was observed in the other steer. It was also observed that the tissues of the bladders had become thin and the inner tissue had extremely injected. Many glassy calculi like rice were found in the bladders.

A urinary calculus about 0.6 cm in diameter was present at the end of the sigmoid flexture of both steers. The hemorrhage was found in the inside of the urethra from the bladder to the end of S-shaped curve. However, the urethra beyond the S-shaped curve was not affected. The lodging of urinary calculus at the S-shaped curve would cause dysuria, which might lead to urolithiasis.

Desquamated epithelium cells of the kidney may form the nuclei of urinary calculi. However, further studies would be necessary to determine whether the histological damage of the kidney is the cause of calculi formation or not.

CHAPTER 2 CHEMICAL COMPOSITION OF URINARY CALCULI IN FATTENING CATTLE

It has been reported in foreign countries that various kinds of urinary calculi having different chemical composition were found in hervivorous animals. Law[52] described eight types of calculi from cattle, classifying them according to their appearance and chemical composition. The four types are mainly calcium carbonate with varying amount of magnesium ammonium phosphate, siliceous and carbonate types. Blout[7] lists seven types of calculi in cattle.

As a preliminary step to study the etiology of urinary calculi, the chemical composition of calculi must be determined. The present experiment was conducted to determine the chemical composition of calculi found in fattening steers in Japan and to study the difference in shape and chemical composition among calculi in various urinary organs.

Materials and Methods

The samples of urinary calculi were collected from animals kept at several places of western Japan as shown in table 2-1. Calculi in the prepuce, crystals found in the prepuital hair and the precipitates in the urine were collected from Wagyu fattening steers and bulls. Fresh urine were left stand for

an hour and filtered to get precipitates. Urinary calculi from the kidney, the bladder and the urethra were collected from two Wagyu fattening steers which were used in the preliminary experiment and those of analytical data were shown in table 2-2.

Table 2-1 Sampling places and urinary organs from which urinary calculi were collected

	Bladder	Urethra	Prepuce	Prepuital hair	Precipitates in urine
Aichi pref. institute of animal industry	1			1	
Hyogo pref. livestock station	1			6	
Gifu pref. livestock station	1	2			
Tottori pref. livestock station				2	
A farm in Hiroshima pref.	1		3	3	
Farm-stock of Kyoto Univ.				6	2
Total	4	2	3	18	2

Urinary calculi were dried at 105 C for five hours and then total nitrogen was determined by the micro-Kjeldal method. After measuring total ash, phosphorus was determined by the method of Fiske and Subbarow[29]. Calcium, magnesium, sodium and potassium were determined by atomic absorption spectroscopy. Carbonic, oxalate, uric acid, xanthin, cystine and cholesterol in calculi were analyzed qualitatively. The presence of polysaccharide was

checked by the PAS staining method and the presence of protein by the xanthoprotein reaction.

The macroscopic and microscopic observation on the appearance of calculi and precipitates in the urine were made. Some of calculi were examined by the PAS staining method.

Results

The chemical composition of urinary calculi and urine precipitates are shown in table 2-2.

Table 2-2 Composition of urinary calculi and precipitates in urine

	Bladder	Urethra	Prepuce	Prepuital hair	Precipitates in urine
No. of samples	4	2	3	18	2
Dry matter %	82.75	78.00	62.84	57.72	-
Total ash %*	73.17	72.47	79.91	78.50	60.07
Nitrogen %	1.76	1.62	5.55	5.83	
Calcium %**	2.73	0.30	0.32	1.02	0.03
Phosphorus	24.63	21.83	25.19	24.80	11.29
Magnesium	19.83	16.32	19.01	19.31	17.42
Sodium	0.37	0.41	0.91	1.05	0.81
Potassium	4.88	5.49	9.00	10.35	19.00
Silica	0.14	0.17	0.13	0.11	0.08

%* : percentage to dry matter.

%**: percentage to total ash.

Dry matter of urinary calculi from the bladder and the urethra was about 80 %, of which approximately 73 % was ash. Phosphorus amounted to 23 % of ash and it was the largest component of minerals. Following phosphorus content, magnesium content was larger component of minerals. Potassium content was 5 to 10 % of ash, and calcium content varied from 0.3 to 8 % of ash.

The composition of urinary calculi obtained from the bladder was similar to that of the urethra, and the component of calculi in the prepuce was similar to that of the prepuital hair. Calculi from the bladder and the urethra had higher content of dry matter and significantly lower contents of ash and nitrogen than those in the prepuce and the prepuital hair. Sodium and potassium contents of ash were significantly lower in the former than the latter.

There appeared to be little difference among locations and between steers and bulls in the chemical composition of urinary calculi. Therefore, most of urinary calculi from fattening cattle in Japan seemed to be made of magnesium phosphate. When compared with precipitates of urine, urinary calculi contained higher total ash and phosphorus, and lower levels of potassium.

In the qualitative analysis, oxalate, cystine and cholesterol were not found, but carbonate, protein and polysaccharide were observed in urinary calculi. Uric acid and xanthine were not found in calculi from the bladder, but observed in calculi from

the prepuital hair.

Table 2-3 indicates the analytical result of calculi from two stteers used in the preliminary experiments (chapter 1).

Table2-3 Mineral composition of urinary calculi

		Kidney	Bladder	Urethra
Total ash	%*	78.90	80.18	83.12
Calcium	%**	0.17	0.08	0.35
Phosphorus		24.95	27.54	27.36
Magnesium		21.38	22.55	24.24
Sodium		5.82	2.98	2.50
Potassium		4.95	4.92	4.47

%* : percentage to dry matter

%**: percentage to total ash

Total ash and each mineral contents of these urinary calculi were similar to those shown in table 2-2. The chemical composition of urinary calucli from the kidney was similar to that of the bladder and the urethra except that sodium content of the former was about twice as much as that of the latter.

Characteristics of urinary calculi of the kidney appeared to be different from those of the bladder and the urethra. The calculi from the kidney were somewhat hard and sand-like, and showed large irregular concretions (fig. 2-1). Most of calculi in the bladder were hard, globular and glassy like rice. The calculi in the urethra were whitish and globular, about 0.5 to

0.6 cm in diameter, and consisted of small particles similar to those found in the bladder. The calculi in the prepuce and prepuital hair were gray-whitish color and were fragile.

Under the microscopic observation, calculi from the kidney had indeterminate shapes. Most of micro calculi from the bladder were like semitransparent stones (fig. 2-2). The large calculus from the bladder had many layers in it and the layers were also found by the PAS staining method (fig. 2-3). Small crystals of triangle prism were found in the precipitates of the urine (fig. 2-4). The same many crystals as found in the precipitates of the urine and a few granular crystals were found in microscopic pictures of calculi from the prepuce and the prepuital hair (fig. 2-5). PAS positive materials of red-purple color were always found around calculi from the prepuce and the prepuital hair.

Discussion

It was reported in the USA, Canada, Australia and New Zealand that calculi from grazing cattle and sheep were mainly made of silica[2,5,17,59]. Woodruff[102] reported urinary calculi consisted of calcium phosphate. It was also reported in the USA that phosphorus and magnesium were the main components of calculi in cattle and sheep which were full-fed concentrate rations, such as milo[13,21,57,64,73].

Phosphorus and magnesium were the main components of urinary calculi in this experiment. Calcium content in calculi was generally low, and silica was a minor component. Therefore, urinary calculi from fattening cattle in Japan may be magnesium-phosphate type.

The mineral composition of urinary calculi of the kidney was little different from those of calculi in the bladder and the urethra. Calculi of the kidney contained larger amount of sodium than those of the bladder and the urethra. The reason for this is not known yet, but the process of calculi formation in the kidney may differ from those of the bladder and the urethra.

The mineral composition of urinary calculi in the bladder and the urethra was similar. It was found that calculi in the urethra consisted of small calculi similar to calculi found in the bladder. Therefore, it was considered that calculi in the bladder moved into the urethra with the urine, lodged at the narrow curvature of urethra, and formed calculi in the urethra.

Calculi in the prepuce and the prepuital hair had higher nitrogen content than those of the bladder and the urethra. Therefore, ammonium might be one of the components of calculi in the prepuce and the prepuital hair. Cornelius et al.[14] reported that fine crystals of triangle prism found in calculi from the prepuce and the prepuital hair were ammonium magnesium phosphate. The dry matter percentage of calculi in the prepuce and the prepuital hair were lower than those of the bladder and the

urethra. The apparent differences in gross appearance and microscopic observation were found between the former and the latter. Therefore, it was suggested that calculi in the prepuce and the prepuital hair were ammonium magnesium phosphate type and calculi in the bladder and the urethra were magnesium phosphate type.

The chemical composition of calculi in the prepuce was similar to that of the prepuital hair. The apparent structual difference between calculi in the prepuce and the prepuital hair was not found under the microscope. Therefore, the process of calculi formation in the prepuce would be similar to that of the prepuital hair.

Next to phosphorus and magnesium, potassium was found to be considerably high in urinary calculi. Potassium content was higher in calculi of the prepuce and the prepuital hair than those of the bladder and the urethra. Urine precipitates contained higher level of potassium than urinary calculi. In other experiments of this study, it was found that the ratio of potassium to phosphorus and magnesium was considerably higher in the urine than in calculi. Therefore, it was considered that the increase of potassium content in calculi was due to the contamination of calculi by the urine.

A small amount of sodium was found in urinary calculi. As similar to the case of potassium, calculi in the prepuce and the prepuital hair contained larger amount of sodium than those of

bladder and the urethra. This may be caused by urine sodium.

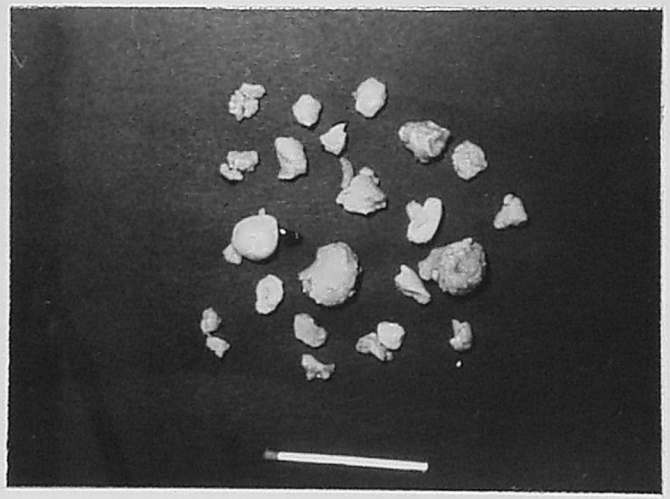


Fig. 2-1 Urinary calculi from the kidney.

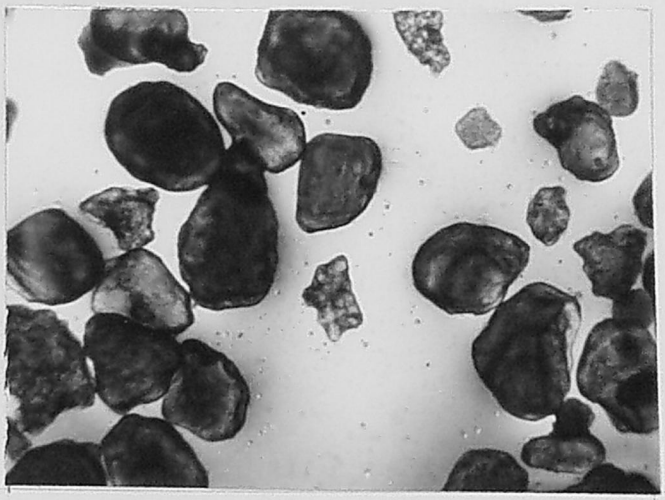


Fig. 2-2 Microscopic picture of micro calculi from the bladder.

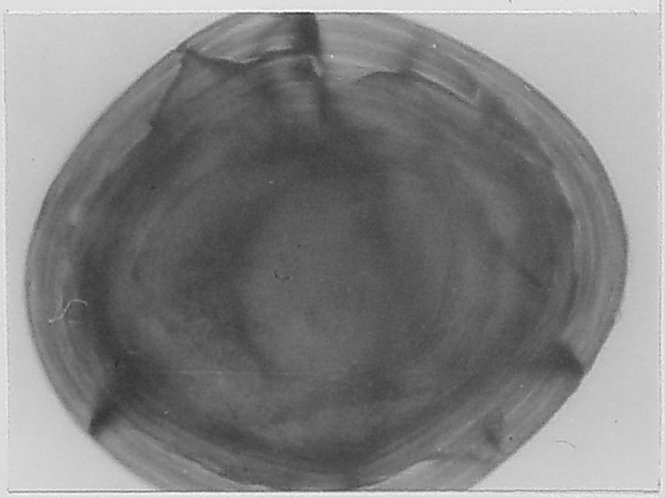


Fig. 2-3 Microscopic picture of a large calculus from the bladder.

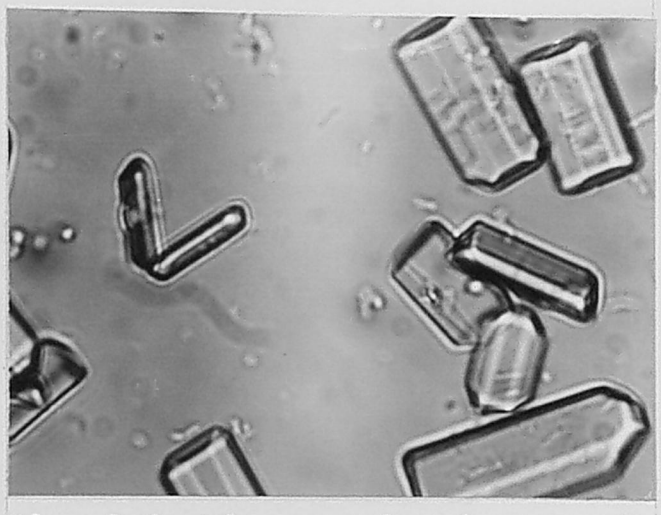


Fig. 2-4 Microscopic picture of the precipitates in urine.

Fig. 2-5 Microscopic picture of urinary calculi from the prepuce.

Summary

This experiment was conducted to determine the chemical composition of urinary calculi found in fattening cattle in Japan and to study the differences in shape and chemical composition among calculi obtained from various organs.

Urinary calculi were collected from the kidney, the bladder, the urethra, the prepuce and the prepuital hair. Ash occupied 70 % of dry matter of urinary calculi and the major components of ash were phosphorus and magnesium. Therefore, urinary calculi found in fattening cattle in Japan may be principally magnesium-phosphate type.

Sodium content of urinary calculi obtained from the kidney was about twice higher than those of calculi found in the bladder and the urethra. Dry matter contents of calculi obtained from the bladder and the urethra were significantly higher, and nitrogen contents were lower than those of calculi from the prepuce and prepuital hair. Sodium and potassium contents in ash of the former group were significantly lower than those of the latter.

In microscopic observation, crushed calculi of the kidney showed indeterminate shapes, and most of micro calculi found in the bladder looked like semitransparent stones. The larger calculus from the bladder consisted of many layers. Layers

were also found by PAS staining method. Many crystals of triangular prism and a few granular crystals were found in calculi of the prepuce and prepuital hair.

Since chemical compositions and microscopic pictures of calculi from the kidney, the bladder, the urethra, the prepuital hair are different, the various process of calculi formation can be expected for each calculi.

CHAPTER 3 RELATIONSHIP BETWEEN URINE AND SERUM MINERAL LEVELS AND INCIDENCE OF URINARY CALCULI IN FATTENING STEERS

The occurrence of urolithiasis in steers is often found at the end of fattening period when high concentrate rations are given. If the formation of urinary calculi is induced by the concretion of urine minerals, the concentration of various urine minerals may change when urolithiasis occures in an animal.

This experiment was conducted to examine the changes of mineral levels in the urine and the serum throughout the fattening period and to determine the relationship between the mineral levels of the urine and the serum and the occurrence of urolithiasis in steers.

Materials and Methods

Fifteen castrated Wagyu calves were divided into two groups, and were fed for 10 months. Feeding period, initial and final body weight, daily gain and feed consumption of the animals were shown in table 3-1.

In A group, concentrate ration was liberally and roughage was fed about 2 kg daily throughout the fattening period and in B group, concentrate ration was given approximately 1.3 % of the

Table3-1 Experimental design and feeding performance

Group	A			B
Steer No.	1	2	3	4-15
Feeding days	295	283	283	328
Initial body weight, kg	212	217	222	213
Final body weight	544	500	516	450
Daily gain	1.13	1.00	1.04	0.72
Feed consumption				
Concentrate	1944	1660	2012	1406
Roughage (dry matter)	586	637	593	1562

body weight and roughage was full-fed. The concentrate ration of both groups consisted of the following : ground corn 45 %, barley 25 %, wheat bran 15 %, rice bran 7 %, soybean meal 6 %, sodium chloride 1 %, calcium carbonate 1 %. The chemical composition of the concentrate ration was the following : 0.36 % calcium, 0.49 % phosphorus, 0.22 % magnesium, 0.43 % sodium and 0.86 % potassium.

The roughage were mainly soiling corn, soiling grass and grass hay. Water was available at all times. Hormone treatment was done, that is, 60 mg of hexesterol dicapricate was injected at 3 and 6 months after the beginning of the experiment.

Blood samples were obtained by jugular vein puncture once a month. Urine samples were obtained twice a week in A group and

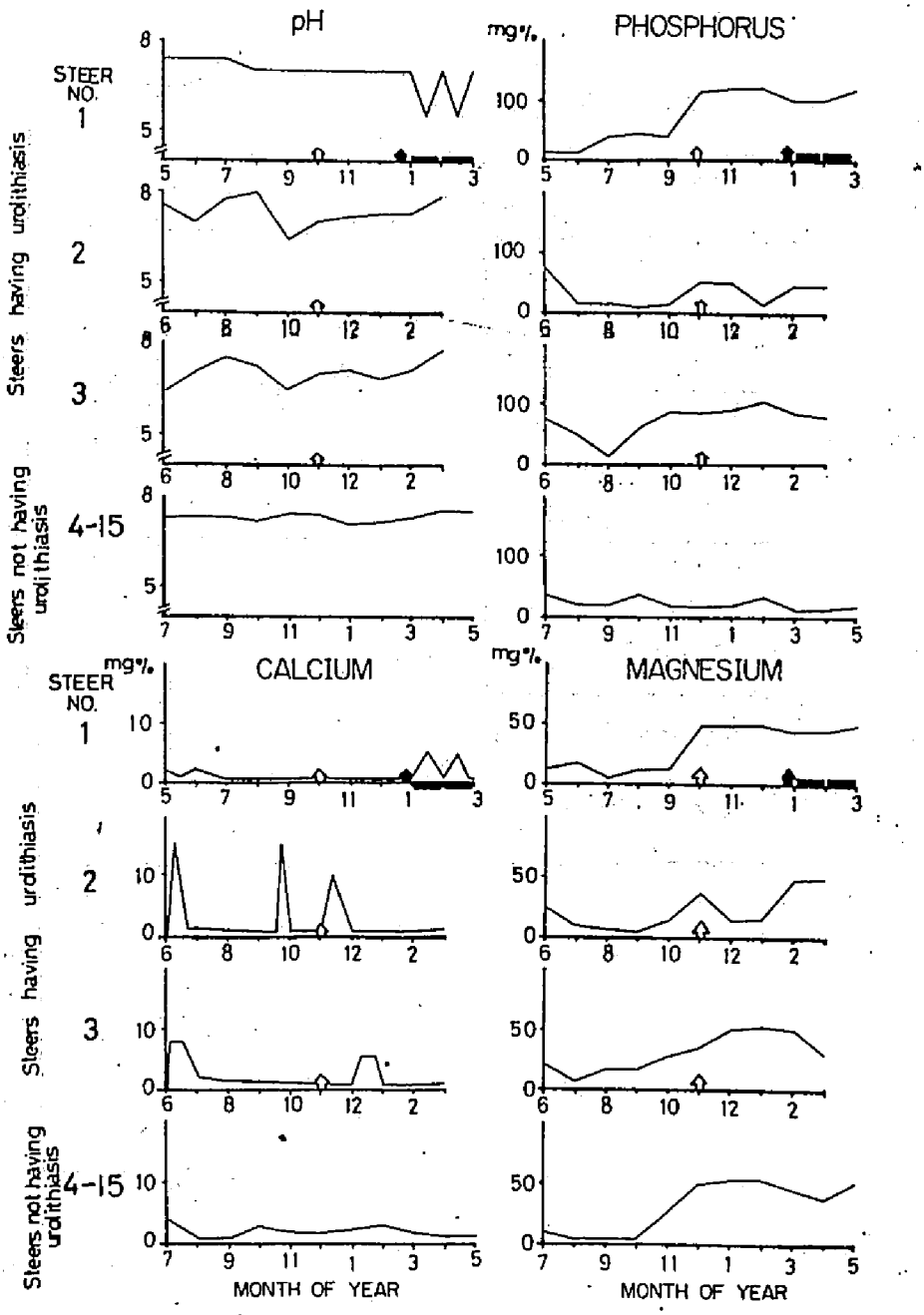
twice a month in B group. After the pH values of urine samples were determined, a few drops of acetic acid and toluen were added to the samples and stored in the stocker at -20°C until analyzed. Blood samples were also stored in the stocker.

Urine pH values were determined by glass rod pH meter. Calcium, magnesium, sodium and potassium of the urine and the serum samples were determined by atomic absorption spectrometer. Urine and serum phosphorus were determined by the method of Fiske and Subbarow[17], urine chloride by the method of Volhard-Harvey, urine mucoprotein by the method of Anderson-Maclagan[1].

Results

As shown in table 3-1, the initial body weight was similar between A and B group. The final body weight and daily gain were larger in A group than in B group. Throughout the fattening period, A group consumed more concentrate ration than B group, and had one third of roughage volume fed to B group.

Urolithiasis occurred in all steers of A group. Especially steer No. 1 had the most serious urolithiasis among them. Whitish crystals were found in the prepuital hair in early fall and the urination of this animal stopped in winter. Ammonium chloride, 40 g daily, was given to the animal several times. In steer No. 2 and No. 3 of A group, whitish crystals were also found in the prepuital hair in mid-fall, but these animals were



⇧: This sign signifies when urinary calculi were found at the prepuital hair.

↑: This sign signifies when inability to urinate was observed.

■: This sign signifies when ammonium chloride was given.

Fig. 3-1-a : Changes of urine components during the fattening period

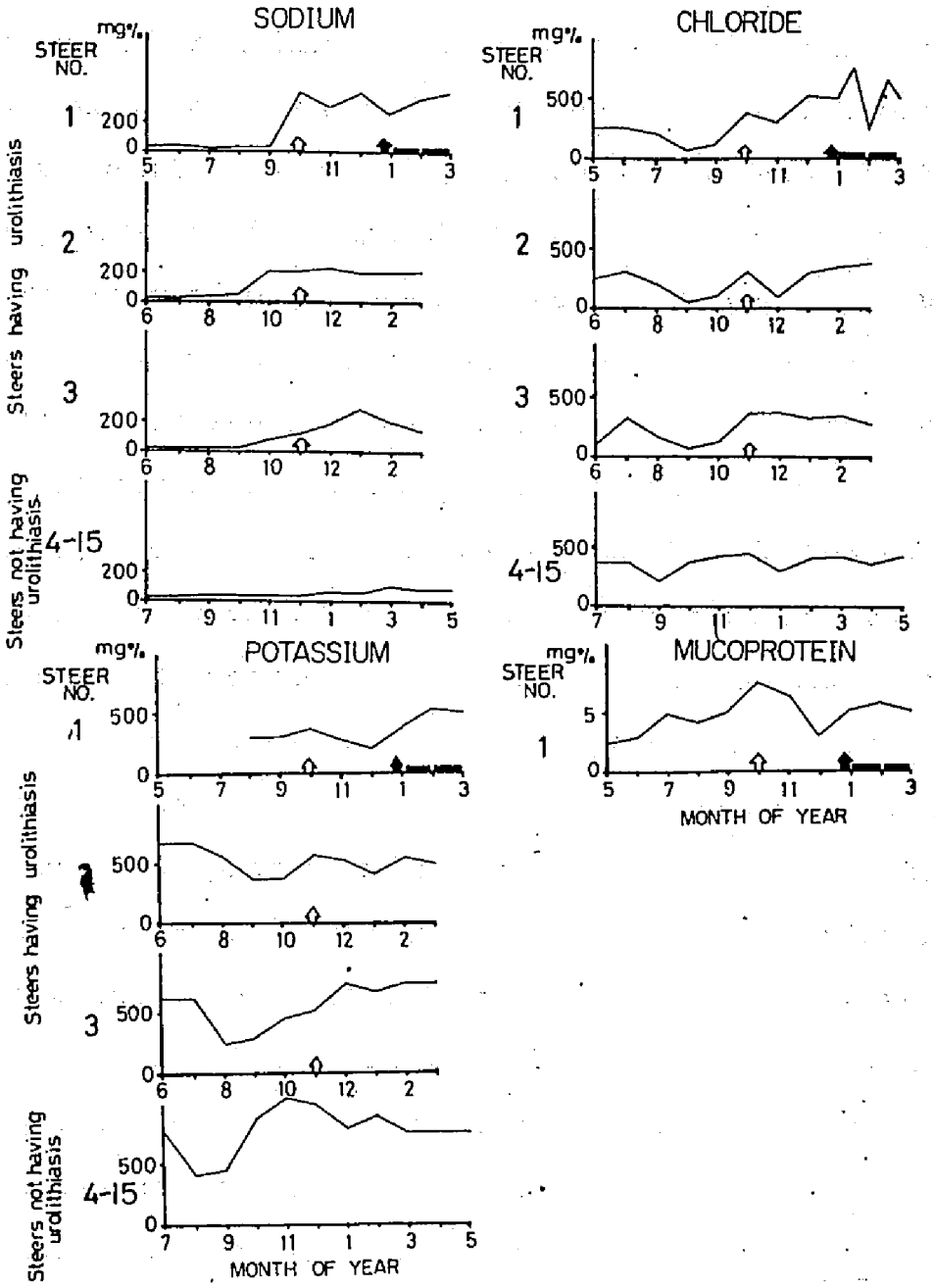


Fig. 3-1-b : Changes of urine components during the fattening period

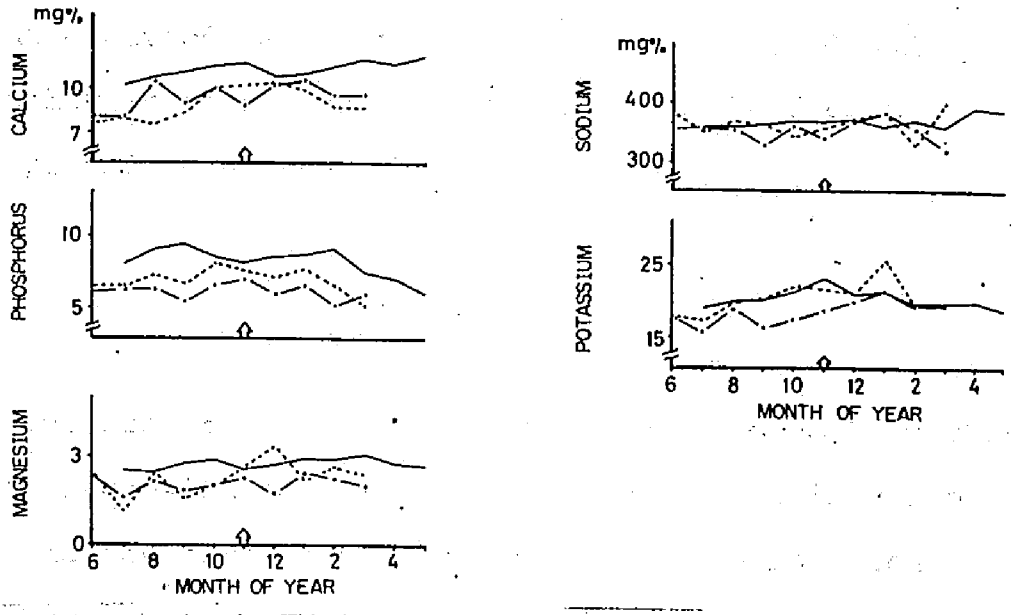
not suffered from serious urolithiasis during the fattening period. Twelve steers of B group did not show a sign of urolithiasis at all throughout the fattening period. Therefore, all the data of B group were combined and the mean values of the group were used.

The data of mucoprotein, mineral and the pH values of the urine were shown in figure 3-1. No clear differences of the urine pH values were found between the beginning and the end of the fattening period and between A and B groups. The urine pH values of steers showed around 7.0 throughout the fattening period.

Urine calcium levels of all steers remained considerably low, but its sudden increase was observed occasionally. No apparent differences of urine calicum level were found between the initial and the final period and between A and B group.

Urine phosphorus, magnesium and sodium levels tended to increase at the latter half of the fattening period in steers suffered from urolithiasis. However, urine phosphorus and sodium levels remained low throughout the experiment period in steers of B group. Urine magnesium levels increased at the latter half of the fattening period in B group.

There was no clear trend for urine potassium and chloride levels but they seemed to decrease slightly in summer. Urine potassium level of A group was always lower than that of B group. When ammonium chloride was given to steer No. 1, the urine pH value decreased and urine calicum and chloride levels increased. The changes of serum mineral levels were shown in figure 3-2.



⇧ : This sign signifies when urinary calculi were found at the prepuital hair.
.....: Mineral concentration of steer No. 2, having urolithiasis.
——: Mineral concentration of steer No. 3, having urolithiasis.
——: Mineral concentration of steer No. 4–15 not having urolithiasis.

Fig. 3-2 : Changes of serum mineral levels during the fattening period

Calcium, phosphorus and magnesium levels of the serum samples from A group were lower than those of B group throughout the fattening period. Serum calcium levels increased and phosphorus levels decreased during the latter half of the fattening period in B group.

Discussion

From the results mentioned above, it was found that urine phosphorus, magnesium and sodium levels tended to increase during the latter half of the experiment in steers which developed urolithiasis. The increase of these minerals may be due to the increased intake of these minerals from rations, and may be due to the abnormal mineral metabolism. There were no apparent trends for urine calcium, potassium and chloride for the entire fattening trial in all steers. However, urine potassium and chloride decreased a little in summer. This may be due to the increased water intake by animals which causes the increase of the urine volume in summer.

For steers not having urolithiasis, urine phosphorus and magnesium levels were high. However, urine potassium levels were low in steers suffered from urolithiasis. Some workers[10,57,64,73] reported that the high urine phosphorus level would be the most important measurable factor associated with the incidence of phosphatic urinary calculi in lambs. As mentioned in the chapter

2, the main components of urinary calculi were phosphorus and magnesium. The increased urine phosphorus level may be the most important factor to induce the formation of calculi.

Cornelius et al.[13] indicated that high levels of phosphorus and sodium were present in the urine of lambs given calculi-provoking ration. However, it was reported that feeding of sodium chloride to animals was an effective means to prevent calculi formation, which increased the urine sodium levels.[11,22,89,90] Therefore, the increase of urine sodium level may not be the main cause of the formation of urinary calculi. The urine potassium levels of steers having urolithiasis were low, but this may not have a direct relation to the incidence of urolithiasis.

Packett et al.[64] reported that urine magnesium levels were high in animals fed calculi-provoking rations. Although urine magnesium levels increased during the latter half of the fattening period, there seemed to be no clear relationship in urine magnesium level between animals having urolithiasis and animals not having it. Therefore, the high level of urine magnesium may not be the main inducing factor to calculi formation, but one of the factors to cause calculi.

Newsom[61] and Frank et al.[30] indicated that the occurrence of urolithiasis in sheep and cattle depended on urine pH. Crookshank et al.[15] also reported that alkaline urine seemed more conducive to phosphatic calculi than acidic urine. On the other hand, Lindley et al.[57] postulated that there was no

apparent relationship between the occurrence of urolithiasis and the levels of urine pH.

In this experiment, the urine pH values of steers having urolithiasis were the same as those of steers not having it. It was found in a series of this study when urine pH was increased, the amount of urine precipitates increased. However, it had been well known when ruminants were fed the high level of grain, urine pH decreased and the occurrence of urolithiasis increased. Therefore, the occurrence of urolithiasis in ruminants may not be associated with the variation of urine pH.

Packett et al.[65] reported that the higher level of urine protein, mainly mucoprotein, were found in lambs which developed urolithiasis than those in lambs which did not develop. However, the urine mucoprotein level was not evidently different between the beginning and the end of the fattening period.

Calcium, phosphorus and magnesium levels in the serum were lower in steers having urolithiasis than in steers not having it. The data of Bushman et al.,[8,9] Robbins et al.[73] and Packett et al.[64] indicated that the amount of serum phosphorus was greater than that of serum calcium in sheep having urolithiasis. However, the amount of serum calcium was greater than that of serum phosphorus in all steers having urolithiasis in this experiment. The difference between the amount of serum calcium and that of serum phosphorus was similar in steers which developed urolithiasis than in steers which did not develop urolithiasis.

Kunkel et al.,[51] Packett and Hauschild[64] and Crookshank et al.[15] reported that serum magnesium levels had been higher in sheep having urolithiasis than in sheep not having it. Johnson et al.[45] indicated that the occurrence of urolithiasis had not been found in lambs which plasma magnesium levels had been increased threefold. In this experiment, the clear relationship was not found between the serum magnesium level and the occurrence of urolithiasis.

Summary

This experiment using fifteen steers was conducted to examine the changes of mineral levels in the urine and the serum throughout the fattening period and to determine the relationship between the mineral concentration in the urine and the serum and the occurrence of urolithiasis.

Urolithiasis occurred during the latter half of fattening period in steers given high concentrate ration, but did not occur in steers given enough roughage. Phosphorus, magnesium and sodium in the urine increased during the latter half of fattening period in all steers. The incidence of urolithiasis was frequently observed when high concentration of phosphorus and sodium and low concentration of potassium were found in the urine. The pH value of experimental animals were about 7.0 throughout the fattening period and seemed to have no relationship to the incidence of urolithiasis.

The difference between calcium and phosphorus levels in the blood serum was smaller in steers having urolithiasis than in steers not having it. Magnesium , sodium and potassium levels in the serum had no apparent relationship to the occurrence of urolithiasis. It is suggested that the high level of urine phosphorus may be one of the most important factors for the calculi formation.

CHAPTER 4 EFFECT OF FEEDING HIGH CONCENTRATE RATIONS ON MINERAL METABOLISM AND INCIDENCE OF URINARY CALCULI

SECTION 1 Effect of Feeding High Concentrate Rations on Urine and Serum Mineral Levels in Wethers

It was observed in a previous experiment that urine concentrations of phosphorus, magnesium and sodium increased and urolithiasis had occurred during the latter half of fattening period in steers given high concentrate rations. But these changes had not occurred in steers given enough roughage. Hawkins[33] indicated the higher the level of concentrate to the level of roughage, the greater the incidence of urolithiasis. Intake of high concentrate ration may bring the special effects on the mineral metabolism in ruminants.

The study in this chapter was conducted to determine the effect of high concentrate rations on mineral metabolism and then examine the relationship between mineral metabolism and physiological changes in rumen.

Materials and Methods

This experiment consisted of two trials. In trial 1, six wethers, averaging about 35 kg, were fed for 67 days in spring. In trial 2, six wethers, averaging approximately 43 kg, were fed for 67 days in summer. Wethers were given

four different concentrate to roughage ratio consisting of following in percent ; 90:10, 80:20, 70:30, 60:40, in each of four periods of twenty days. The composition of basal ration is shown in table 4-1.

When a high concentrate ration is abruptly given, the effect on mineral metabolism may be different from that in the case which a high concentrate ration is gradually increased. Therefore six wethers were alloted two groups with three wethers each in both trials. One group was given from 90:10 to 60:40, and the other group from 60:40 to 90:10. The ration was given twice daily at the level of 1 % of the body weight, and water was available at all times. The mineral contents of ration are shown in table 4-2.

After thirteen days of preliminary period, urine, serum and fecal samples were obtained during the next seven days in each four periods. Urine samples were collected under toluene and urine pH values were determined for each 24-hr collection. Fecal samples were dried prior to storage. Blood samples were obtained by jugular vein puncture twice a sampling period.

Results

The results of mineral intake, excretion and retention in both trials are shown in table 4-3.

Table 4-1 Composition of basal ration

	Concentrate		Roughage
Trial 1	Composition		
	Flaked corn	90 %	Rice straw
	Soybean meal	10 %	
	Mineral supplement		
	Calcium carbonate	1 % of the ration	
	Sodium chloride	1 % of the ration	
Trial 2	Composition		
	Barley	75 %	Alfalfa hay cube
	Soybean meal	25 %	
	Mineral supplement		
	Calcium carbonate	1 % of the concentrate	
	Sodium chloride	1 % of the concentrate	

Table 4-2 Dietary mineral contents

	Concentrate-roughage ratio 1)			
	60:40	70:30	80:20	90:10
Trial 1				
Calcium	0.51	0.50	0.48	0.45
Phosphorus	0.21	0.21	0.21	0.21
Magnesium	0.12	0.12	0.11	0.11
Sodium	0.54	0.54	0.53	0.53
Potassium	1.07	0.96	0.87	0.77
Trial 2				
Calcium	0.95	0.88	0.82	0.75
Phosphorus	0.38	0.40	0.41	0.43
Magnesium	0.33	0.30	0.27	0.24
Sodium	0.49	0.52	0.54	0.57
Potassium	0.81	0.75	0.68	0.62

Minerals are represented as percentage of dry matter.

1) : % of air dry matter

Table 4-3-1 Effect of concentrate to roughage ratio on mineral balances (Trial 1)

	Concentrate to roughage ratio[1)]			
	60:40	70:30	80:20	90:10
Calcium, g per day				
Intake	3.81	3.65	3.33	2.88
Urinary excretion	0.01	0.01	0.01	0.04
Fecal excretion	1.45	0.95	0.71	0.92
Retention	2.35	2.69	2.61	2.02
Phosphorus, g per day				
Intake	1.49	1.48	1.43	1.30
Urinary excretion	0.06	0.11	0.17	0.24
Fecal excretion	1.48	1.43	1.39	1.35
Retention	-0.05	-0.06	-0.13	-0.29
Magnesium, g per day				
Intake	0.84	0.82	0.78	0.69
Urinary excretion	0.33	0.33	0.27	0.27
Fecal excretion	0.52	0.50	0.45	0.42
Retention	-0.01	-0.01	0.06	0.00
Sodium, g per day				
Intake	4.03	3.96	3.71	3.35
Urinay excretion	2.94	2.71	2.88	2.30
Fecal excretion	0.45	0.58	0.34	0.26
Retention	0.64	0.67	0.49	0.79
Potassium, g per day				
Intake	7.83	7.20	6.11	4.82
Urinary excretion	4.71	4.33	3.36	2.34
Fecal excretion	0.65	0.65	0.38	0.36
Retention	2.47	2.22	2.37	2.12

1) : % of air dry matter

Table 4-3-2 Effect of concentrate to roughage ratio on mineral balances (Trial 2)

	Concentrate to roughage ratio[1)]			
	60:40	70:30	80:20	90:10
Calcium, g per day				
Intake	8.21	7.61	7.09	6.49
Urinary excretion	0.08	0.07	0.06	0.05
Fecal excretion	2.59	2.24	1.95	1.64
Retention	5.54	5.30	5.08	4.80
Phosphorus, g per day				
Intake	3.29	3.46	3.35	3.72
Urinary excretion	0.34	0.57	0.72	0.69
Fecal excretion	2.36	2.46	2.43	2.12
Retention	0.59	0.43	0.40	0.91
Magnesium, g per day				
Intake	2.85	2.59	2.33	2.08
Urinary excretion	0.76	0.69	0.58	0.53
Fecal excretion	1.34	1.18	1.03	0.74
Retention	0.75	0.72	0.72	0.81
Sodium, g per day				
Intake	4.24	4.50	4.67	4.93
Urinary excretion	2.34	2.87	3.03	3.02
Fecal excretion	0.23	0.29	0.23	0.27
Retention	1.67	1.34	1.41	1.64
Potassium, g per day				
Intake	7.00	6.49	5.88	5.36
Urinary excretion	7.53	6.57	5.93	5.59
Fecal excretion	0.38	0.47	0.45	0.38
Retention	-0.91	-0.55	-0.50	-0.61

1) : % of air dry matter

The fecal calcium excretion and the calcium retention tended to be parallel to the amount of calcium intake in both trials. In comparison with the fecal excretion, the urine calcium excretion was very low. The urine excretion averaged about 1.5 and 3.0 % of total excretion in each trials. The urine calcium excretion tended to be increased slightly in higher concentrate rations in trial 1, while it was decreased a little extent in higher concentrate rations in trial 2.

The fecal excretion and the retention of phosphorus were approximately parallel with changes of phosphorus intake in trial 1. But, in both trials, the urine phosphorus excretion increased in higher concentrate rations and was not parallel the phosphorus intake. The phosphorus intake was evidently lower in trial 1 than in trial 2. And negative phosphorus retentions were found in trial 1. The urine phosphorus excretion falled into the range from 3.9 to 15.1 % of total excretion in trial 1 and the range from 12.6 to 24.6 % in trial 2.

The fecal magnesium excretion and the urine magnesium excretion were parallel to the amount of magnesium intake, and the magnesium retention appeared to be no obvious difference among four treated rations in both trials. The mean urine magnesium excretion was 38.1 % of total excretion in trial 1 and 37.7 % in trial 2.

In comparison with the fecal excretions of sodium and

Table 4-4 Effect of concentrate to roughage ratio on urine volume, pH values and mineral levels in wethers

	Concentrate-roughage ratio [1]			
	60:40	70:30	80:20	90:10
Trial 1				
Urine values, mg/100ml				
Calcium	1.0	1.2	1.4	7.0
Phosphorus	12	21	36	65
Magnesium	58	65	56	57
Sodium	574	560	604	531
Potassium	925	880	725	528
Chloride	1039	1053	1062	877
Urine volume, ml	575	535	525	492
Urine pH	8.3	8.4	8.1	7.3
Trial 2				
Urine values, mg/100ml				
Calcium	4.8	3.6	3.8	2.8
Phosphorus	17	40	49	36
Magnesium	42	48	37	31
Sodium	129	182	217	213
Potassium	401	473	437	367
Chloride	390	410	412	435
Urine volume, ml	2529	2056	2100	2335
Urine pH	8.2	8.7	8.5	8.0

1) : % of air dry matter

potassium, the urine excretions of these two minerals were extremely higher. In trial 1, 87.2 % of sodium was excreted via urine and 91.8 % of sodium in trial 2. The urine potassium excretion was 87.9 % of toal excretion in trial 1 and 93.8 % in trial 2. The urine excretions of these two minerals were parallel to the intakes of sodium and potassium respectively in two trials. There seemed to be no obvious difference among treated rations in the retentions of sodium and potassium in two trials. Negative potassium retentions were found in trial 2.

Urine mineral concentrations in trial 1 and 2 are shown in table 4-4. The urine calcium level was apparently increased in wethers given the highest concentrate ration in trial 1. On the contrary, the urine calcium concentration tended to be slightly decreased as the concentrate to roughage ratio increased in trial 2. The urine phosphorus level tended to be increased in high concentrate rations in trial 1 and 2. Particularly, the increasing of urine phosphorus was more evident in trial 1 than in trial 2. In high concentrate to roughage ratio, the decreasing of urine potassium level was found in both trials.

No difference in urine magnesium, sodium and chloride was obviously found among four rations in two trials. In comparison with trial 2, urine levels of magnesium, sodium, potassium and chloride were higher in trial 1. The lower pH values in urine were found in the highest concentrate ration in trial 1, while, in trial 2, there was no difference in urine pH among treated rations. The urine volume was decreased to a little extent in higher

concentrate to roughage ratio in trial 1, while there appeared to be no obvious difference in the urine volume among four rations in trial 2. The urine volume was markedly more in trial 2 than in trial 1. This difference may be due to the season in trials were conducted.

Serum data from trial 1 and 2 are shown in table 4-5. Serum levels of calcium and potassium tended to be decreased to a little extent in higher concentrate rations in both trials. Serum phosphorus levels appeared to be increased in high concentrate to roughage ratio in trial 1, while no difference was found among four treated rations in trial 2.

Table 4-5 Effect of concentrate to roughage ratio on serum mineral levels in wethers

(mg/100ml)

	Concentrate-roughage ratio 1)			
	60:40	70:30	80:20	90:10
Trial 1				
Calcium	9.2	9.0	9.0	8.7
Phosphorus	6.1	6.6	7.0	7.0
Magnesium	2.9	2.9	3.2	3.0
Sodium	306	316	271	322
Potassium	25	26	24	23
Trial 2				
Calcium	9.9	10.0	9.6	9.5
Phosphorus	7.5	7.4	8.1	7.6
Magnesium	3.1	3.3	3.4	3.3
Sodium	332	337	336	340
Potassium	25	24	23	23

1) : % of air dry matter

Discussion

From the results of this experiment, it was generally found that there was a close relationship between the amount of intake and the urine mineral excretion and the serum mineral level. But, in trial 1, it was observed in wethers given the high concentrate ration that the urine calcium level and excretion, the urine phosphorus level were not parallel to the amount of each mineral intake. However, these trends were not found in wethers of trial 2 given the ration which contained the higher level of protein.

When wethers were given the high level of concentrate ration, urine pH were lowered in trial 1. This result was in accordance with the report of Reed et al.[71] that a mild form of acidosis was observed in cattle given the high concentrate diets and the acidosis was manifested by a high titratable acidity. On the other hand, urine pH was not decreased in wethers of trial 2. The cause was considered as following that rumen pH was heightened by increasing ammonia production when a high protein ration was given in trial 2.

It was found that urine calcium levels were increased in wethers given the higher level of concentrate ration in trial 1. Lennon et al.[54] reported that glucose ingestion had caused acidification of the urine and had increased urine calcium excretion in man. As previously reported[10,11,58], urine calcium were markedly increased and urine pH were lowered when

ruminants were administered ammonium chloride. The increased urine calcium excretion would be owing to metabolic acidosis which was induced by ammonium chloride. Lemman et al.concluded in their report[53] that metablic acidosis produced a sharp increase of urine calcium excretion by causing decreased urine calcium reabsorption in the kidney.

The clear increases of urine phopshorus level and of urine phosphorus excretion were found in wethers which were fed with the higher level of concentrate which was high-energy and low-protein ration in trial 1. This result was in accordance with the report of Ricketts et al.[72] and Reed et al.[71].

It was suggested by Pitts et al.[68] that changes in acid load were effectively compensated by changes in titratable acid and ammonia excretion when renal function was normal in non-ruminant. The results in this experiment suggested that acids produced by giving high concentrate rations are excreted, in most part, as free titratable acid, namely, phosphate ion. On the other hand, Scott et al.[77] showed that all of acid were excreted as ammonium ions when hydrochloric acid was infused into the rumen. It was also found in chapter 7 that supplement of ammonium chloride increased the urine phosphorus level in samll extent although it lowered the urine pH. It may be considered from these findings that the kidney may excrete acid largely in combination with ammonia when acids are loaded as inorganic acids, such as, hydrochloric acid or ammonium chloride.

In trial 2, the urine phosphorus level and excretion were increased to lesser extent than in trial 1 when a high concentrate ration was given. The urine pH was not so different among four treated rations. Since the dietary phopshorus content was slightly increased and the dietary calcium was decreased when the concentrate and roughage ratio was heightened in trial 2. Increased urine phosphorus level and excretion would be, in part, owing to the lowered dietary calcium to phosphorus ratio.

The urine levels of magnesium, sodium and potassium were higher in trial 1 than those in trial 2. This difference might be due to the difference of urine volume between both trials. Since potassium intake in trial 2 was not so different from the value in trial 1, the reason why negative retentions were shown in trial 2 was not known.

Since the heightened urine calcium level may not be associated with the formation of urinary calculi, other changes induced by the high concentrate ration must be considered in relation to calculi formation. An acid urine seems to prevent the formation of urinary calculi. And a high level of urine phosphorus should be the most important factor in calculi formation as discussed in chanpter 3. It may be considered that there may be boundary line of calculi formation between urine phosphorus level as aninducing factor and urine acidity as a preventive factor. If the inducing factor is stronger than the preventive factor, phosphatic urinary calculi would be formed. Adversely, if urine

acidity is superior to the increasing of urine phosphorus level, urinary calculi would not be formed.

In addition, early work with rats by Eaton[20] indicated that the addition of an excess of acid or basic sodium phosphate to the food was followed by extensive pathological changes in the kidney. It is also conceivable that the increase of urine phosphorus concentration induced by a high concentrate ration may become a cause of renal pathological changes and an inducing factor of urolithiasis.

Summary

The experiment of this section was conducted to study the effect of giving high concentrate rations on the urine and serum mineral levels and mineral metabolism and to examine an inducing factor of calculi formation in wethers.

The experiment was consisted of two trials. In both trials animals were given four different ratio of concentrate to roughage consisting of following in percent ; 90:10, 80:20, 70:30, 60:40,. The concentrate ration in trial 2 contained higher protein level than in trial 1.

In trial 1, urine pH values were lowered and the urine calcium and phosphorus levels and serum phosphorus levels elevated as the ratio of concentrate to roughage increased, and these changes were not parallel to the amount of these mineral intakes. The increased urine phosphorus concentration induced by a mild acidosis may become one of causative factor to produce urinary calculi.

In trial 2, urine pH values were not changed and a close relationship between the intake and the excretion of minerals with the exception of phosphorus were found as the concentrate to roughage ratio increased. Urine concentration and excretion of phosphorus were only increased to a small extent when a higher concentrate ration was given. The increase of urine phosphorus would be owing to lowered dietary calcium to phosphorus ratio.

SECTION 2. Relationship Between Urine Mineral Levels and Rumen Fermentation in Wethers Fed with High Concentrate Ration

It was shown in the experiment of chapter 4, section 1 that urine pH values had been lowered and the urine calcium and phosphorus levels had been elevated by giving high level of high-energy and low-protein concentrate ration to wethers. However there is little information about a cause why changes of urine mineral levels were induced by giving a high concentrate ration.

It is well established that excessive grain consumption by ruminants causes overingestion, acute ingestion, diarrhea and lactic acidosis[18,19,100]. And it is also well known that volatile fatty acid and lactic acid productions in the rumen are changed by giving a high concentrate ration[19,74,87,88,100].

This experiment was conducted to study the cause of changes of urine mineral levels through rumen fermentation when a high concentrate ration was given in wethers.

Materials and Methods

Six wethers, averaging about 35 kg, were fed with four different rations of which concentrate to roughage ratio consisting of the following percentages ; 90:10, 80:20, 70:30, 60:40, in each four periods of twenty days.

Six wethers were alloted two groups with three wethers each. One group (A) was given from 90:10 to 60:40, and the other group (B) from 60:40 to 90:10. The ration was given twice daily at the level of 1 % of the body weight, and water was available at all times. The composition of basal ration and the chemical data of the ration are shown in table 4-6 and 4-7.

After preliminary thirteen days, rumen and serum samples were obtained during the next seven days in each four periods. Urine samples were collected everyday, and rumen and serum samples once a sampling period. Rumen and serum samples were collected twice a sampling day, (1) just before the morning feed, (2) at three hours after the morning feed. Rumen samples were taken by stomach tube and the pH value of rumen fluid was immediately determined with pH test paper. Rumen samples were preserved with mercuric chloride for volatile fatty acid analysis.

The volatile fatty acids were analyzed as free fatty acids by gas chromatograph, and total content of volatile fatty acids according to the method of Conway[43]. Lactic acid concentrations were determined by the method of Baker and Summerson[4]. Blood samples were obtained from jugular vein and collected into tubes containing heparin sodium. Blood sugar concentrations were determined by the method of Somogyi and Nelson[82].

Table 4-6 Composition of the basal ration

	Concentrate		Roughage
Composition			
	Flaked corn	90 %	Rice straw
	Soybean meal	10 %	
Mineral supplement			
	Calcium carbonate	1 % of the ration	
	Sodium chloride	1 % of the ration	

Table 4-7 Chemical analysis of the basal ration

	Corn flake	Soybean meal	Rice straw
Dry matter (%)	86.9	90.3	91.4
Organic matter	85.5	84.0	80.7
Crude protein	9.1	33.5	4.5
Crude fat	2.3	1.6	2.0
Nitrogen free extract	73.1	45.4	49.3
Crude fiber	1.1	3.5	25.0
Crude ash	1.4	6.2	10.0
Energy (K cal/g)	11.1	4.7	3.8

Results

The results of urine calcium, phosphorus and magnesium concentrations are shown in table 4-8. Urine calcium and phosphorus concentrations were significantly higher ($p<.05$) in the highest concentrate to roughage ratio than in the lowest concentrate to roughage ratio in A group. The urine phosphorus level in B group was increased to a little extent with heightening the concentrate to roughage ratio, but this trend was not significant. Urine pH values were significantly decreased ($p<.05$) with increasing the high concentrate in both groups.

The results of pH value in rumen fluid are shown in table 4-9. Rumen pH values of post-feeding were significantly lower ($p<.01$), as compared with those of pre-feeding. Although a noticeable change was not found in rumen pH values of pre-feeding, pH values of post-feeding were significantly decreased ($p<.05$) with heightening the concentrate to roughage ratio in both groups. A substantial difference in pH values was not found between A and B group.

The data of total VFA content and VFA composition in rumen fluid were shown in table 4-10 and 4-11 respectively.

Table 4-8 Effect of concentrate to roughage ratio on urine mineral levels and pH values

(mg/100ml)

Group		Concentrate-roughage ratio[1)] 60:40	70:30	80:20	90:10
A	Urine Calcium	1.1[a]	1.4[a]	1.8	12.8[b]
	Phosphorus	11[a]	29	49	110[b]
	Magnesium	79	72	61	56
	pH	8.0[a]	8.2[a]	8.4[a]	7.2[b]
B	Urine Calcium	1.0	1.0	1.0	1.1
	Phosphorus	12	13	23	21
	Magnesium	38	58	51	59
	pH	8.7[a]	8.6[a]	8.0	7.5[b]

a and b : Means with different superscript letters differ significantly ($p<.05$).

1) : % of air dry matter

Table 4-9 Effect of concentrate to roughage ratio on pH values in ruminal fluid

	Concentrate-roughage ratio[1)] 60:40	70:30	80:20	90:10
Pre-feeding mg/100ml				
A	6.9	6.8	6.9	7.1
B	7.0	6.9	6.9	7.0
3 hr after feeding				
A	6.1	6.3[a]	5.6[b]	5.5[b]
B	6.3[a]	6.3[a]	5.7[b]	5.7[b]

a and b : Means with different superscript letters differ significantly ($p<.05$).

1) : % of air dry matter

Table 4-10 Effect of concentrate to roughage ratio on total VFA concentration in ruminal fluid

(mM/100ml)

	Concentrate-roughage ratio [1)]			
	60:40	70:30	80:20	90:10
Pre-feeding				
A	49.7	44.7	45.7	42.3
B	46.1 [a]	39.1	28.7	32.1 [b]
3 hr after feeding				
A	57.0 [a]	55.9 [a]	62.1	83.2 [b]
B	73.1	64.7	62.1	64.2

a and b : Means with different superscript letters differ significantly ($p<.05$).

1) : % of air dry matter

The concentrations of total VFA were significantly higher ($P<.01$) in post-feeding than those in pre-feeding. In ruminal fluid of pre-feeding, a significant difference was not found in A group, but, in B group, the total VFA concentration in the highest concentrate ration was significantly lower ($p<.05$) than that in the lowest concentrate ration. Adversely in ruminal fluid of post-feeding, a significant increase ($p<.05$) was found in A group, while a certain tendency was not observed in B group with increasing the concentrate ration. When the ration containing 60 % concentrate was given, the total VFA concentration of

Table 4-11 Effect of concentrate to roughage ratio on VFA composition in ruminal fluid

(mol %)

		Concentrate-roughage ratio 1)			
		60:40	70:30	80:20	90:10
Pre-feeding					
A	C_2	66.2^{a}	61.9	55.8^{b}	56.0^{b}
	C_3	18.9	21.4	26.7	24.9
	C_4	11.1	11.9	12.9	11.1
	C_{i5}	2.7	3.5	3.8	3.3
	C_5	1.2^{a}	1.5	3.5^{b}	4.3^{b}
	C_2/C_3	3.5^{a}	2.9	2.1	2.3^{b}
B	C_2	65.2^{a}	64.4^{ac}	56.9^{bc}	56.4^{b}
	C_3	20.0	18.0	25.6	25.8
	C_4	10.2	12.0	11.6	10.3
	C_{i5}	3.3	4.0	3.8	4.9
	C_5	1.5	1.8	2.5	2.8
	C_2/C_3	3.3	3.6	2.2	2.2
3 hr after feeding					
A	C_2	65.3^{a}	59.2	52.8^{b}	52.6^{b}
	C_3	20.9	24.4	27.4	21.5
	C_4	11.6^{a}	13.1^{ac}	15.2^{bc}	18.9^{b}
	C_{i5}	1.4	1.3	0.7	1.1
	C_5	0.8^{a}	1.3^{c}	4.6^{b}	5.9^{b}
	C_2/C_3	3.1	2.4	1.9	2.4
B	C_2	62.6	63.5	54.1	62.3
	C_3	23.8	19.4	28.5	18.9
	C_4	10.9	14.0	13.5	14.9
	C_{i5}	1.4	1.6	0.5	0.6
	C_5	1.4	1.6	3.0	3.4
	C_2/C_3	2.6	3.3	1.9	3.3

a, b and c : Means with different superscript letters differ significantly ($p<.05$).

1) : % of air dry matter

post-feeding was significantly lower ($p<.05$) in A group than in B. On the contrary , when the highest concentrate ration was given, the total VFA concentration in post-feeding was, although not significant, higher in A than in B.

A significant difference between pre-feeding and post-feeding was not found in a molar percentage of VFA except iso-valeric acid. As the concentrate ration increased, the molar percentage of acetic acid was significantly ($p<.05$) decreased in pre-feeding of both groups. Although the same trend was observed in post-feeding of A group, a certain tendency was not found in B group. When the highest concentrate ration was given, the difference between A and B group was significant ($p<.05$) in acetic acid.

Though the difference was not significant, a molar percentage of propionic acid in pre-feeding tended to be increased as increasing the concentrate ration. However a constant tendency was not observed in propionic acid of post-feeding. The molar percentage of butyric acid of pre-feeding was not significantly affected by varying the concentrate to roughage ratio. A constant trend was not also found in butyric acid of A group in post-feeding, while the significant increase ($p<.05$) was found in B group with increasing the concentrate ration. When the ration containing 90 % concentrate was given, the difference between

A and B was significant ($p<.05$) in butyric acid.

As the concentrate ration increased, the molar percentage of valeric acid in pre-feeding and post-feeding was significantly ($p<.05$) increased in A group, while no significant increase was found in B group. The difference of C_2/C_3 ratio of pre-feeding in A group was significant between the groups given 60 % and 90 % concentrate.

The concentration of lactic acid in rumen fluid is shown in table 4-12. The lactic acid concentration in post-feeding were significantly higher ($p<.01$) than those in pre-feeding. Though the slight increase was observed in post-feeding as increasing the concentrate ration, this was not significant. Particularly, this trend appeared to be clearer in A than in B.

The lactic acid in blood plasma were shown in table 4-13. An obvious difference was not observed between pre-feeding and post-feeding, between A and B group and among treated rations.

Table 4-14 shows single correlation coefficients between the concentrations of urine calcium, phosphorus and magnesium, and the VFA in rumen fluid. The urine calcium was significantly correlated with the molar percentage of butyric acid ($r=0.462$, $p<.05$), the valeric acid ($r=0.499$, $p<.05$), the total VFA ($r=0.440$, $p<.05$), the rumen pH ($r=-0.437$, $p<.05$) and the urine pH ($r=-0.656$, $p<.01$). The phosphorus had a high relationships with the molar percentage of acetic acid ($r=-0.479$, $p<.05$), the butyric acid ($r=0.652$, $p<.05$), the valeric acid ($r=0.842$, $p<.001$), the total

VFA ($r=0.436$, $p<.01$), the lactic acid ($r=0.646$, $p<.01$), the rumen pH ($r=-0.640$, $p<.01$) and the urine pH ($r=-0.643$, $p<.01$).

Table 4-12 Effect of concentrate to roughage ratio on lactic acid level in ruminal fluid

	Concentrate-roughage ratio			
(% of air dry matter)	60:40	70:30	80:20	90:10
Pre-feeding mcg/ml				
A	3.9	4.5	8.2	4.3
B	2.3	6.2	8.0	5.4
3 hr after feeding				
A	9.9	16.6	21.3	20.3
B	9.0	16.8	14.6	13.7

Table 4-13 Effect of concentrate to roughage ratio on lactic acid level in blood plasma

	Concentrate-roughage ratio			
(% of air dry matter)	60:40	70:30	80:20	90:10
Pre-feeding mcg/ml				
A	51	56	64	66
B	72	78	62	58
3 hr after feeding				
A	66	92	72	72
B	56	99	69	58

Table 4-14 Correlation coefficients between urine minerals and rumen VFA

	Urine mineral levels		
	Calcium	Phosphorus	Magnesium
Rumen fluid			
C_2	-0.352	-0.429*	-0.161
C_3	-0.159	-0.233	0.006
C_4	0.462*	0.652**	0.098
C_5	0.499*	0.842***	-0.122
C_2/C_3	-0.032	-0.085	-0.363
Total VFA	0.440*	0.436*	0.027
Lactic acid	0.288	0.646**	-0.055
pH	-0.437*	-0.640**	0.017
Urine pH	-0.656**	-0.643**	0.033

Discussion

Dunlop and Hammond[19] reported rumen concentration of approximately 160 mM/L of L-lactate and 60 mM/L of D-lactate and pH values between 4.0 and 4.5 in a steer engorged with corn. Dirksen[18] also observed an increase of blood lactate reaching maxima of total lactate 240 mg% during the first phase of acute acidosis. The degree of acidosis which was found in this study was considered to be mild, since the lowest mean pH value in rumen fluid was 5.5 and the highest mean level of rumen lactic acid was 21.3 mg%.

When 90 % of concentrate ration was given, the significant increase of urine calcium and phosphorus levels were found only in A group as the ratio of concentrate to roughage increased. The increase of lactic acid and total VFA concentrations in rumen fluid was more in A group than in B. And VFA composition was significantly affected only in A group as increasing the ratio of concentrate, that is, a molar percentage of acetic acid was decreased and that of butyric acid was increased. The differences of urine mineral levels between A and B group may be owing to those of rumen VFA.

Telle et al.[87] reported that the excretions of urine calcium had been markedly increased whithin 3 hrs. post infusion of racemic solution of D-L lactic acid intraruminally, followed by a rapid decrease over the next 2 hrs.. Dirksen[18] also observed the increase

of urine and serum phosphorus concentrations during the acidosis. Therefore, the increase of lactic acid level in rumen fluid seems to have surely a positive association to the elevation of urine calcium and phosphorus levels.

Since the increase of rumen lactic acid concentration was a small amount in this experiment, other factors also may affect the elevation of urine calcium and phosphorus levels. Topps et al.[88] observed the increase of urine phosphorus without any increasing of rumen lactic acid concentration when the high concentrate ration was given to cattle. Dunlop[19] reported that an exponental decline in the concentration of lactate had been observed after the addition of sodium lactate to the rumen of cattle in a dose of 11.4 mM/kg. Dirksen[18] also postulated that the lowering of rumen pH was not caused by an increase in lactic acid concentration when the acidosis was less severe form.

The molar percentage of butyric acid, valeric acid, total VFA, the rumen pH and the urine pH had high correlations with the urine calcium and phosphorus. However, the molar percentage of acetic acid and the rumen lactic acid was significantly associated with the urine phosphorus only. Therefore it is conceivable that urine levels of calcium and phosphorus may be affected, in part at least, by different causes in wethers given a high concentrate ration.

The lactic acid level in blood plasma was not clearly changed when the concentrate to roughage ratio increased.

Huber et al.[42] reported that injected L-lactate had a half-life of 22 min., and injected D-lactate had a half-life of approximately 90 min.. It might be resulted from a rapid disappearance of lactic acid from blood plasma that there was no difference in lactic acid levels in blood plasma.

Summary

This experiment was conducted to study the cause of changes of urine mineral levels through rumen fermentation in wethers fed with a high concentrate ration.

Animals were given the ration of four different ratio of concentrate to roughage consisting of the following percentages ; 90:10, 80:20, 70:30, 60:40,. One group (A) was given from 90:10 to 60:40, and the other group (B) from 60:40 to 90:10.

Urine calcium and phosphorus were increased in A group by giving the highest concentrate ration. The rumen pH value lowered in both groups, but the total VFA in rumen fluid increased only in A group as increasing the ratio of concentrate. The rumen lactic acid level, although not significant, increased in both groups, while this trend was more obvious in A group than in B. Molar percentages of acetic acid, butyric acid and valeric acid in rumen fluid of post-feeding were significantly changed only in A group with varying the concentrate to roughage ratio.

Many VFA were highly correlated with the urine calcium and phosphorus concentrations, but some VFA were significantly associated with the urine phosphorus only. Therefore it is conceivable that urine calcium and phosphorus may be affected, in part at least, by different causes in wethers given a high concentrate ration.

CHAPTER 5 EFFECT OF DIETARY CALCIUM AND PHOSPHORUS LEVELS ON MINERAL METABOLISM AND INCIDENCE OF URINARY CALCULI

SECTION 1 Effect of Addition of Calcium Carbonate on Mineral Metabolism and Incidence of Urinary Calculi in Wethers

In general, a concentrate ration contains more phosphorus and less calcium than a roughage. Especially wheat bran and rice bran, which have been frequently used for fattening cattle in Japan, are rich in phosphorus. When a high concentrate ration is given, it is conceivable that dietary calcium and phosphorus ratio is low.

A high dietary phosphorus level has been shown to induce a high incidence of phosphatic urinary calculi in cattle and sheep[8,16,64]. On the other hand, it was reported that the increase of calcium level in the ration reduced the incidence of calculi[8,9,25,26].

In this chapter, the effect of dietary calcium and phosphorus levels on urine and serum mineral levels and the incidence of urinary calculi was investigated. And then the effect of dietary calcium level on the distribution of minerals in the digestive tract was studied.

This investigation was conducted to determine the influence of different calcium and phosphorus levels on the forming of urinary calculi, urine mineral levels, serum mineral levels and mineral balances in wethers.

Materials and Methods

This experiment was consisted of two trials. The composition of basal rations and its mineral contents are shown in table 5-1 and 5-2. The ration was given twice a day at the level of 1 % of the body weight. Water was offered ad libitum. Wethers were placed in metabolic cages throughout the experimental period.

Urine samples were collected under toluene and urine pH values were determined for each 24-hrs collection. Fecal samples were dried prior to atorage. Blood samples were obtained by jugular vein puncture twice a sampling period. These samples were obtained during the last week of the experimental period in both trials.

Trial 1 : Nine wethers averaging about 42 kg in weight were divided into three groups, three wethers each, and were fed sixty seven days from November to January. The each group was given the following rations which contained 0.1 % calcium and 0.6 % phosphorus, 0.6 % calcium and 0.6 % phosphorus and 1.2 % calcium and 0.6 % phosphorus. The approximate levels of calcium and phosphorus were achieved by adding calcium carbonate to the basal ration. At the end of the sampling period, two wethers in each group were slaughtered for the examination of urinary calculi.

Trial 2 : Six wethers averaging about 53 kg in weight were alloted for two groups, three wethers each, on the basis of weight, and were fed for fifty days from March to May. The one group was given the ration which contained 0.1 % calcium and 1.1 % phosphorus.

The ration of the other group which was supplemented with calcium carbonate contained 1.3 % calcium and 1.1 % phosphorus. At the end of the sampling period, all wethers were slaughtered and the bladder and the kidney were examined for urinary calculi.

Table 5-1 Composition of the basal rations

Ingredients	Trial 1	Trial 2
Ground barley	56 %	40 %
Wheat bran	24	24
Rice bran	-	15
Rice straw	20	20
Sodium chloride	-	1

Table 5-2 Mineral composition of the basal rations

(% of air dry matter)

Minerals	Trial 1	Trial 2
Calcium	0.10	0.10
Phosphorus	0.53	1.13
Magnesium	0.22	0.25
Sodium	0.10	0.39
Potassium	1.00	0.88

Results

At the autopsy, sand-like micro calculi were observed visually in the kidney of one wether given the lowest calcium ration in trial 1 and each one wether in both groups of trial 2 had urinary calculi in the kidney (table 5-3). Urine mineral levels, urine pH and urine volume are shown in table 5-3.

Table 5-3 Effect of various dietary calcium and phosphorus levels on the incidence of urinary calculi and urine mineral levels in wethers

Trial	1			2	
Calcium %	0.1	0.6	1.2	0.1	1.3
Phosphorus %	0.6	0.6	0.6	1.1	1.1
Urinary calculi incidence					
No. of wethers	1	0	0	1	1
Urine values, mg/100 ml					
Calcium	0.6	1.2	1.0	2.7	3.8
Phosphorus	180^{a}	93^{b}	36^{c}	223^{e}	333^{f}
Magnesium	37	45	59	22^{e}	46^{f}
Sodium	104^{a}	119^{a}	247^{b}	176	216
Potassium	503^{a}	493^{a}	845^{b}	450^{e}	582^{f}
Chloride	135	129	171	429	414
Urine volume, ml	784	570	585	970	743
Urine pH	6.89^{a}	7.35^{a}	8.26^{b}	6.86	6.52

a, b and c : Means with different superscript letters differ significantly ($p<.01$).

e and f : Means with different superscript letters differ significantly ($p<.05$).

Urine calcium levels appeared not to be influenced by increasing of the dietary calcium in both trials. Urine phosphorus levels in trial 1 appeared to be lower than those in trial 2. Urine phosphorus levels were significantly lowered ($p<.01$) in trial 1, but were adversely heightened ($p<.05$) in trial 2 by the giving of calcium carbonate. Urine magnesium concentrations were significantly ($p<.05$) increased in higher calcium group of trial 2, but the difference between trial 1 and 2 was not significant.

Urine sodium and potassium concentrations tended to be increased by increasing the dietary calcium content. The difference in urine sodium levels among groups reached significance in trial 1 ($p<.01$), and the increasing of urine potassium was significant in trial 1 ($p<.01$) and in trial 2 ($p<.05$). There was no substantial difference in the urine chloride and the urine volume in both trials.

The urine pH heightened ($p<.01$) when calcium carbonate was supplemented to the ration in trial 1. However, the urine pH in trial 2 was not significantly different between two groups and these values seemed to be considerably low in comparison with in trial 1. Mineral levels of blood serum are presented in table 5-4.

The serum calcium level was increased ($p<.01$) by the supplementation of calcium in trial 1, while the serum calcium level in trial 2 was not increased. The serum phosphorus level

Table 5-4 Effect of various dietary calcium and phosphorus levels on serum mineral levels

Trial	1			2	
Calcium %	0.1	0.6	1.2	0.1	1.3
Phosphorus %	0.6	0.6	0.6	1.1	1.1
Serum values, mg/100 ml					
Calcium	7.7[a]	9.1[b]	9.3[b]	7.0	7.4
Phosphorus	10.8	10.1	8.3	17.9	16.9
Magnesium	3.6	3.7	3.3	4.5[d]	4.2[e]
Sodium	393	402	369	362	347
Potassium	22	21	21	24	25

a and b : Means with different superscript letters differ significantly ($p<.01$).

d and e : Means with different superscript letters differ significantly ($p<.05$).

tended to decrease with the elevation of dietary calcium in both trials, but the difference was not significant. In wethers given higher dietary calcium in trial 2, the serum magnesium level decreased substantially ($p<.05$). There was no significant difference in the serum levels of sodium and potassium in both trials.

The mineral excretion and balances are shown in table 5-5.

Table 5-5 Effect of various dietary calcium and phosphorus levels on mineral balances

Trial	1			2	
Calcium %	0.1	0.6	1.2	0.1	1.3
Phosphorus %	0.6	0.6	0.6	1.1	1.1
Calcium, g per day					
Intake	0.74	3.76	9.16	0.69	12.27
Urinary excretion	0.01	0.01	0.01	0.03	0.03
Fecal excretion	0.66[a]	1.31[b]	2.59[c]	1.09[e]	4.78[f]
Retention	0.07[a]	2.25[b]	6.57[c]	-0.43[e]	7.46[f]
Phosphorus					
Intake	4.39	3.84	4.35	8.78	10.27
Urinary excretion	1.53[a]	0.53[b]	0.26[c]	2.00	2.36
Fecal excretion	2.09	2.22	3.07	3.31	2.30
Retention	0.77	1.09	1.02	3.47	5.61
Magnesium					
Intake	2.23	1.59	2.23	1.92	2.25
Urinary excretion	0.28	0.23	0.26	0.27	0.32
Fecal excretion	0.93	0.80	0.96	0.97	0.95
Retention	1.02	0.92	1.01	0.68	0.98
Sodium					
Intake	2.17	1.88	2.13	3.01	3.52
Urinary excretion	0.81[a]	0.70[a]	1.35[b]	1.38	1.58
Fecal excretion	0.61	0.29	0.23	0.59	0.20
Retention	0.75	0.89	0.55	1.04	1.74
Potassium					
Intake	8.52	7.36	8.66	6.87	8.03
Urinary excretion	3.36[a]	2.72[a]	4.31[b]	4.19	4.26
Fecal excretion	1.24	0.75	0.49	1.10[e]	0.49[f]
Retention	3.92	3.90	3.86	1.57	3.28

a, b and c : Means with different superscript letters differ significantly ($p < .01$) in trial 1.

e and f : Means with different superscript letters differ significantly ($p < .01$) in trial 2.

There was no significant difference in the urine calcium excretion among treatment groups in both trials, while the urine calcium excretion in trial 1 was larger in amount than that in trial 2. The fecal calcium excretion and the calcium retention were significantly increased ($p<.01$) with the supplementation of calcium to the ration in both trials. Negative calicum balances were observed in wethers given the lower calcium ration in trial 2.

As the calcium level in the ration increased, the urine phosphorus excretion decreased significantly ($p<.01$) in trial 1. Though a significant difference was not found, the fecal phosphorus excretion appeared to be increased by giving the high dietary calcium in trial 1. The administration of calcium to the ration appeared to increase the urine phosphorus excretion and to decrease the fecal phosphorus excretion in trial 2, though differences were not significant.

No significant difference in the urine excretion, the fecal excretion and the retention of magnesium was found among treatment groups in both trials, while the increasings of urine magnesium excretion and of the magnesium retention approached significance in trial 2 ($p<.05$).

Urine excretion of sodium and potassium increased ($p<.01$) in wethers given the highest dietary calicum ration in trial 1. In trial 2, the difference in urine excretion of sodium and potassium was not so apparent between two groups as the

case in trial 1. The fecal excretion of sodium and potassium were apparently decreased with the supplementation of calcium carbonate to the ration in both trials. And the difference in the fecal potassium excretion between two groups was significant in trial 2 ($p<.01$). There was no significant difference in sodium and potassium retentions among three groups in trial 1 and between two groups in trial 2.

Discussion

A small amount of urinary calculi was found in the kidney of one wether given 0.1 % calcium ration in trial 1. This suggests that there is the protective effect of giving calcium carbonate on the forming of urinary calculi when the phosphorus level of a ration was 0.6 %. This result was supported by the reports of several workers that calcium carbonate offered some degree of protection of phosphatic urolithiasis[8-11,16-18,24,25].

In the experiment of chapter 3, a positive relationship was observed between the high level of urine phosphorus and the occurrence of urinary calculi. The urine phosphorus level and excretion were decreased by the addition of calcium carbonate to the ration in trial 1 of this experiment. Therefore the efficacy of calcium carbonate on the protection of calculi formation seemed

to be due to the reduction of urine phosphorus level. This view is in accordance with the demonstration of several researchers[10,11,25,26].

In this experiment fecal phosphorus excretion were increased in wethers which were given high dietary calcium ration in trial 1. This result suggested that the addition of calcium to the ration led to the precipitation of unsoluble calcium phosphate and interfered the absorption of phosphate. Decreased urine and serum phosphorus levels caused by adding calcium carbonate would be, in part, owing to the interference of phosphorus absorption.

Gill et al.[31] reported that the proctective effect of calcium carbonate against phosphatic urolithiasis in rats appeared to involve a decreasing of phosphatic absorption from intestine. Leuker et al.[55] and Ricketts et al.[72] indicated that animals given the ration of low calcium to phosphorus ratio absorbed the greater amount of dietary phosphorus than animals given high calcium to phosphorus ratio.

Moreover, it is generally believed that ionized calcium levels in the blood regulates parathyroid hormone secretion. It was also reported that a reduction of urine phosphorus excretion followed the intravenous infusion of calcium in normal men[35,36,41]. The apparent absorption of calcium was strikingly increased and the serum calcium level was clearly increased by giving calcium carbonate in trial 1. It may be possible to consider in wethers given high calcium ration that high serum calcium levels would induce hypoparathyroid state and secondary

hypoparathyroidism reduced urine phosphorus excretions.

On the contrary, urinary calculi were found in the kidney in each one wether of two groups in trial 2 and calcium supplementation was considered not to be effective to prevent urinary calcium. It was also observed in trial 2 that the urine phosphorus level was increased by giving calcium carbonate and these values were extremely high in both groups in comparison with in trial 1. The urine calcium level was higher in trial 2 than in trial 1.

The phosphorus level in the ration used in trial 2 was 1.1 %, and the dietary calcium level in calcium supplemented ration was 1.3 %. Bushman et al.[11] indicated that calcium to phosphorus ratio in a ration should be at least 2:1 in order to prevent urinary calculi and Vipperman et al.[96] also postulated that calcium to phosphorus ratio of 1.3:1 was the borderline to the occurrence of urinary calculi. The result in trial 2 that urinary calculi was observed in a wether given calcium supplemented ration may support these postulation.

The fecal excretion of phosphorus was not increased by giving calcium carbonate in trial 2. Serum phosphorus levels in both groups in trial 2 were exceedingly higher than any other serum phosphorus levels obtained in previous experiments. It may also considerable from these results that phosphorus metabolism may differ from it in animals which are given lower phosphorus ration when animals are given a extremely high phosphorus ration.

Krishnaro et al.[50] reported that a hyperparathyroidism

induced by high dietary phosphorus could not be fully counteracted by increasing the calcium content of the diet because at high intakes of calcium the fraction absorbed was markedly depressed in mice. Handler et al.[34] and Levine et al.[56] believe that an increase in plasma inorganic phosphorus is a further stimulus to the parathyroid glands. A low calcium and a strikingly high phosphorus level in blood serum may induce hyperparathyroid state and this state may not be changed by the addition of calcium to a ration.

Urine pH values were clearly elevated by giving the calcium carbonate in trial 1, while urine pH values were low and were not different between two groups in trial 2. In the reports of South Dakota group [8,9,10,11,38,39], the urine pH value seemed not to be different among sheep given various calcium and phosphorus levels. However, Emerick et al.[27] recently reported that the giving of dibasic sodium phosphate as a phosphorus supplement decreased urine pH in lambs. It can be considered that the increasing of urine pH may be owing to the decreased phosphorus absorption by adding calcium to the ration in trial 1. And no difference in urine pH between two groups in trial 2 may be due to the same amount of phosphorus absorption in two groups.

The addition of calcium to the ration increased the urine magnesium excretion in trial 2. Serum magnesium levels appeared to be lowered in wethers given calcium carbonate in trial 1 and 2. This result was in agreement with the reports of Bushman et

al.[10]. Crookshank et al.[16] and Kunkel et al.[51] reported that a higher level of serum magnesium was associated with the incidence of urianry calculi in lambs. But the serum magnesium concentration was lowered to a little extent and the incidence of urinary calculi was found in a wether which was fed with the high calcium ration. Therefore, a decreased serum magnesium level seemed not to be effective to prevent the formation of calculi.

When calcium carbonate was added to the ration, urine excretion of sodium and potassium were increased and fecal excretion of these minerals appeared to be decreased in both trials. These results were in accordance with the reports of Bushman et al.[10,11]. It would be possible to consider from above results that the supplementation of calcium carbonate to a ration increased the absorption of sodium and potassium.

Since the elevation of urine pH was found in trial 1 and, in addition, the increasing of urine sodium and potassium levels were larger in trial 1 than those in trial 2, it would be suggested that the urine concentrations of sodium and potassium might have the correlation with the urine pH. As previously mentioned (chapter 3), the urine concentrations of sodium and potassium may not have the direct relation to the incidence of urinary calculi.

Summary

The experiment using fifteen wethers was conducted to examine the effect of addition of calcium carbonate on the incidence of urinary calculi and on mineral metabolism.

Calcium carbonate was used to obtain 0.1, 0.6 and 1.2 % calcium, and the phosphorus level in the ration was shown to be 0.6 % in trial 1. The following percentages of calcium ; 0.1 and 1.3, were obtained by the addition of calcium carbonate to the basal ration, and the phosphorus level was shown to be 1.1 % in trial 2.

In trial 1, the incidence of urinary calculi was found in a wether given the lowest dietary calcium. In trial 2, urinary calculi were observed in two wethers which were given low and high dietary calcium respectively.

Urine phosphorus concentrations were decreased by the addition of calcium carbonate in wethers which were fed with 0.6 % phosphorus ration. However, urine phosphorus levels were not decreased by adding calcium carbonate to the basal ration in wethers given 1.1 % phosphorus ration. The addition of calcium carbonate to the ration tended to increase urine sodium and potassium excretions and to reduce fecal sodium and potassium excretion.

The efficacy of calcium carbonate to the protection of forming of urinary calculi was considered to be due to the decrease of urine phosphorus level.

SECTION 2 Effect of Dietary Calcium on Mineral Level and Its Solubility of Digesta in Wethers

It was found in a previous section that urine phosphorus excretion was decreased and urine sodium and potassium excretion were increased by the addition of calcium carbonate to the ration. Gill et al.[31] suggested that excess calcium in the diet resulted in a fall of urine phosphorus because most of the food phosphate was converted in the gut of rats to the non-absorbable calcium phosphate.

This experiment was conducted to examine the effect of various level of dietary calcium on the mineral level and the solubility of digesta in wethers given a high concentrate ration.

Materials and Methods

Six wethers averaging about 35 kg in weight were divided into three groups, two wethers each,. The each group was given the following rations which contained 0.1, 0.6 and 1.2 % of calcium. The ration of all groups contained 0.6 % of phosphorus. The dietary calcium level was adjusted by adding calcium carbonate to the ration. The composition and its mineral contents are shown in table 5-6.

The ration was given twice a day at the level of 1 % of the body weight. Water was offered ad libitum. After sixtyseven days feeding, all sheep were slaughtered at two hours after the

Table 5-6 Composition of basal ration and its mineral composition

Ingredients		Minerals	
Ground Barley	56 %	Calcium	0.10
Wheat bran	24	Phosphorus	0.53
Rice straw	20	Magnesium	0.22
		Sodium	0.10
		Potassium	1.00

morning feed. Polyethylene Glycol 4000 (PEG), as a marker, was given for seven days before slaughter at the level of two percent of concentrate ration. Sodium chloride was not added to the ration. Digesta samples were collected from rumen, omasum, abomasum, upper small intestine, middle small intestine, lower small intestine, cecum and colon. The pH value of digesta was immediately determined after obtaining the samples. Supernatant fluid in digesta was obtained with centrifugation at 12000 rpm for one hour.

Digesta samples were dried at 105°C for five hours and then ashed with dry ashing method. Phosphorus was determined by the method of Fiske and Subbarow [29]. Calcium, magnesium, sodium and potassium were determined with atomic absorption spectrophotometry. The mineral concentration in supernatant corrected with the marker was expressed by the following equation.

$$\frac{\text{Marker (\% of daily oral intake) in 1 g feed (dry matter)}}{\text{Marker (\% of daily oral intake) in 1 g digesta (dry matter)}}$$

X mg mineral in 1 g digesta = mg mineral/ 1 g of feed digesta

The solubility of mineral was determined by the following calculation method. The mineral content and the dry matter were expressed as gram and the mineral solubility was as percentage.

$$\frac{\dfrac{\text{Mineral content of 1 ml supernatant}}{\text{1-dry matter of 1 ml supernatant}}}{\dfrac{\text{Mineral content of 1 g digesta}}{\text{1-dry matter of 1 g digesta}}} \times 100 = \text{Mineral solubility}$$

Results

The pH values in digesta were shown in table 5-7.

Table 5-7 The pH in digesta at different sites of digestive tracts

Ca : P	R	O	AB	S-I	S-II	S-III	CE
0.1 : 0.6	4.9	4.9	3.7	5.6	7.8	8.4	8.1
0.6 : 0.6	5.2	4.9	2.9	5.3	7.2	8.5	8.4
1.2 : 0.6	5.0	4.7	2.4	5.3	7.3	8.5	8.3

The pH of digesta was lowest in abomasum and was highest in lower digestive tract. The pH values in rumen and omasaum were higher than in abomasum. Although the pH value in abomasum appeared to be lowered as dietary calcium increased, its trend was not evident in other sites of digestive tract.

The calcium concentrations in supernatant and digesta, and the calcium solubility are shown in figure 5-1.

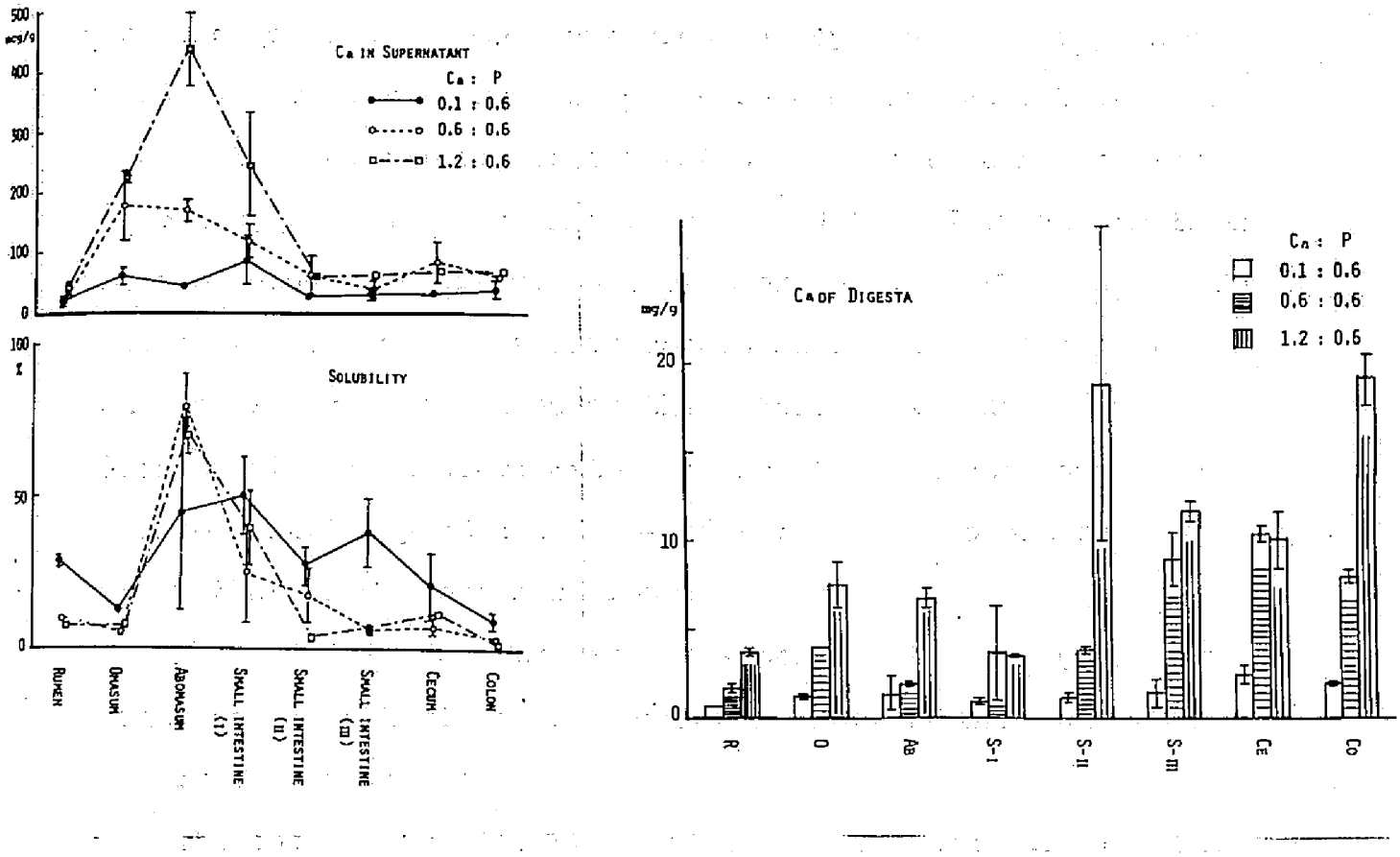


Fig. 5-1 Calcium concentration in supernatant and digesta, and calcium solubility at different sites of digestive tract

In the rations containing 0.6 and 1.2 % calcium, the variation of calcium concentration in supernatant along with digestive tract was similar to the changes of the calcium solubility. The calcium concentration in supernatant and its solubility were highest in abomasum, and then decrease in lower sites of digestive tract. On the other hand, in the lowest calcium ration, the change of calcium concentration in supernatant was not parallel to the change of the calcium solubility. In all sites of digestive tract with the exception of abomasum, the calcium solubility tended to be higher in the lowest calcium ration than in the other rations.

The calcium concentration in supernatant and digesta were heightened in all sites of digestive tract as increasing the dietary calcium level.

The phosphorus concentration in supernatant and digesta, and solubility are shown in figure 5-2. The phosphorus concentration in supernatant was higher in omasum and the upper part of small intestine than in the other sites of digestive tract. No apparent difference in phosphorus concentration in supernatant was found among three groups from rumen to the middle part of small intestine. But, in the lower part of small intestine, cecum and colon, the phosphorus concentration in supernatant was substantially lower in the highest calcium ration than in the other two rations.

Likewise, there is also no constant difference among three groups in the phosphorus solubility from rumen to the middle part of small intestine. From the lower part of small intestine

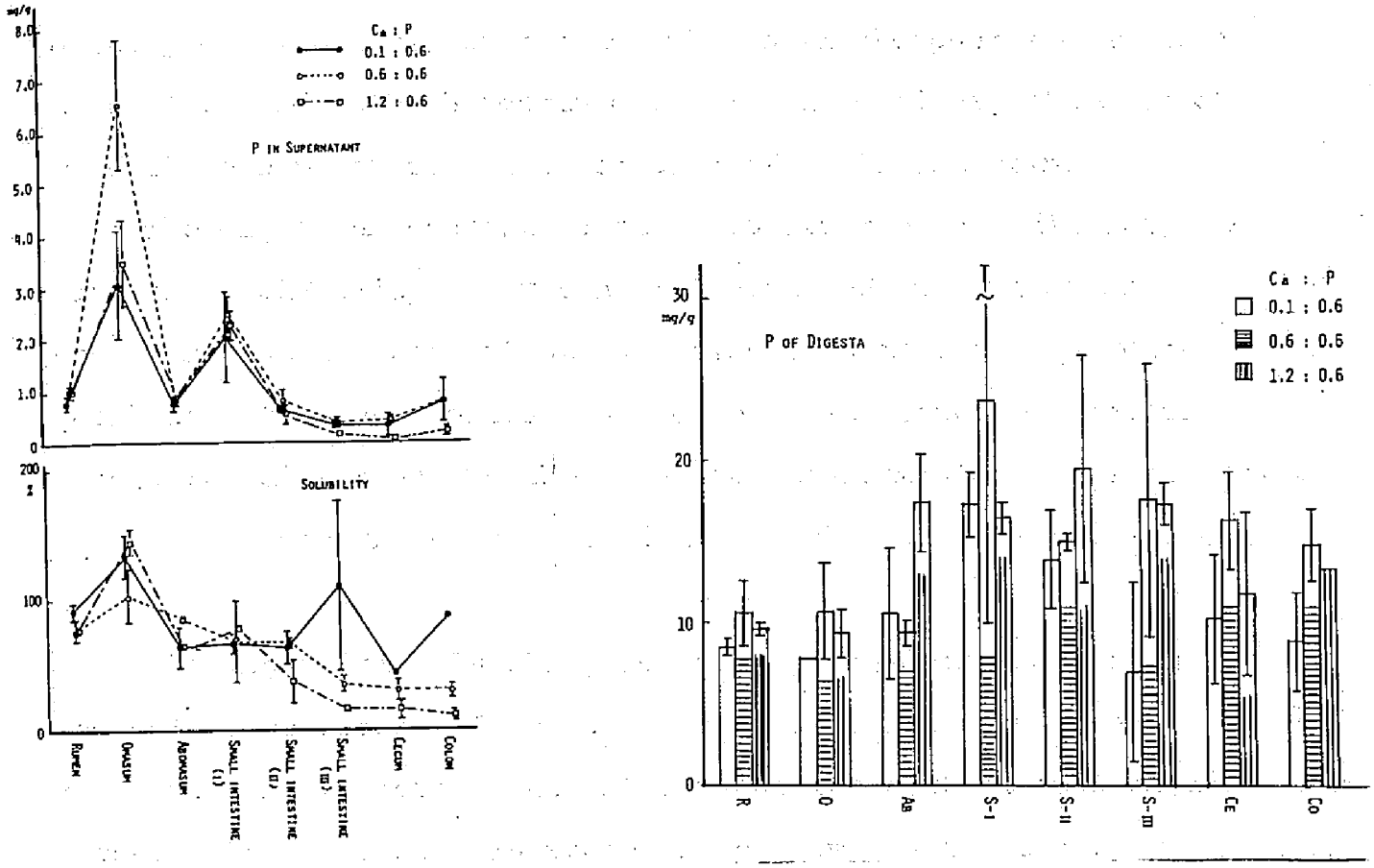


Fig. 5-2 Phosphorus concentration in supernatant and digesta, and calcium solubility at different sites of digestive tract

to colon, however, the phosphorus solubility was lowered as the dietary calcium level increased. The total phosphorus content of digesta tended to be heightened in all sites of digestive tract with the exception of the upper part of small intestine.

The magnesium concentration in supernatant and digesta, and magnesium solubility are shown in figure 5-3. The magnesium concentration in supernatant was heightened in the upper part of small intestine and lowered in the following sites of digestive tract. The difference in the magnesium concentration in supernatant among treatment groups was not found in all sites of digestive tract.

The magnesium solubility appeared to be decreased in omasum and colon. No clear difference in the solubility was observed among three treatment groups. The total magnesium content in digesta appeared to be lowered in rumen and omasum, on the contrary, to be heightened in the lower part of small intestine and colon as increasing the dietary calcium level.

The sodium concentration in supernatant and digesta, and sodium solubility are shown in figure 5-4. The sodium concentration in supernatant was heightened in omasum and the upper part of small intestine. Among three treatment groups, there appeared to be no clear difference in sodium concentration in supernatant. Although the variation of sodium solubility was so wide that an evident trend was not clear, the mean value of sodium solubility tended to be increased from the lower part of small intestine to cecum.

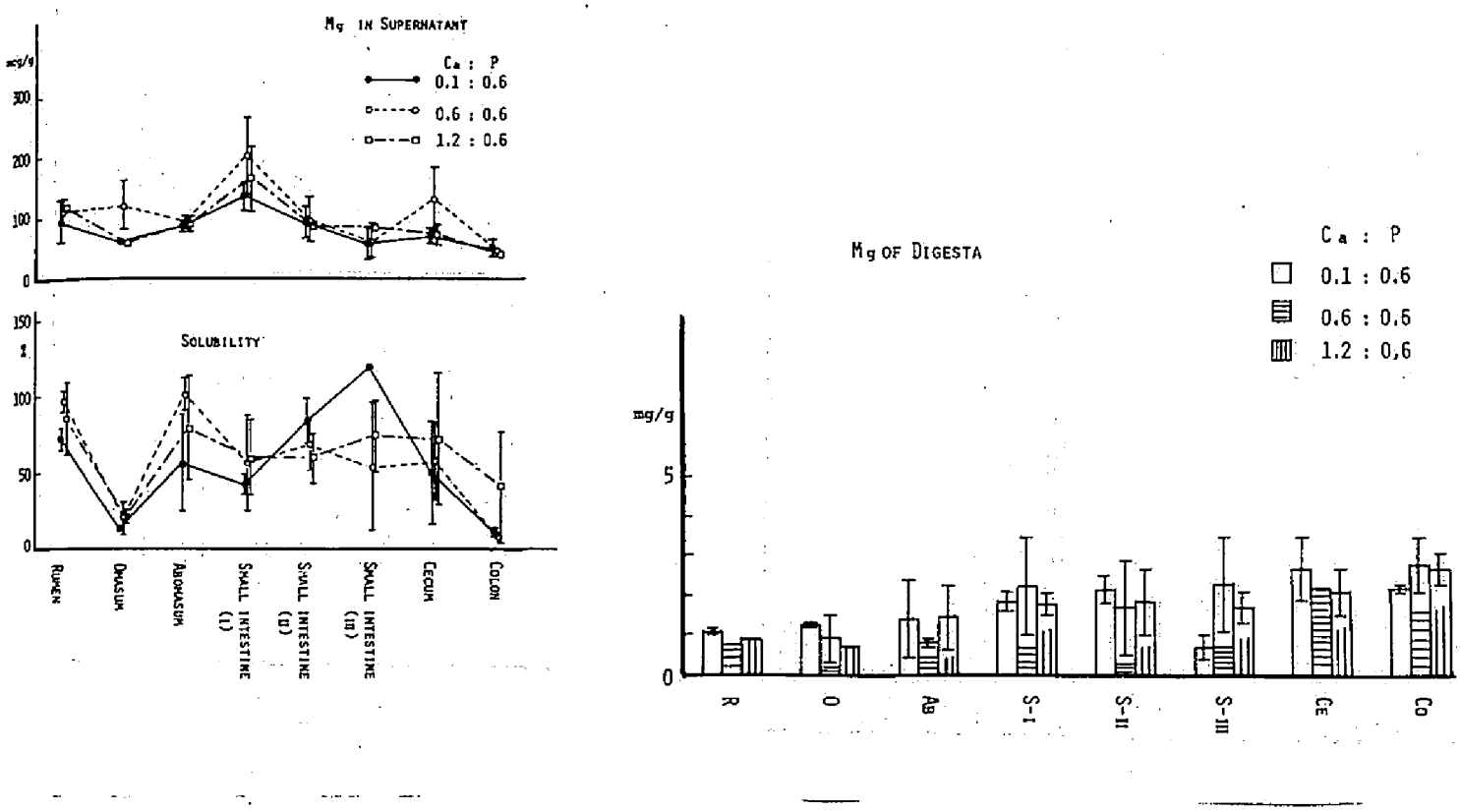


Fig. 5-3 Magnesium concentration in supernatant and digesta, and magnesium solubility at different sites of digestive tract

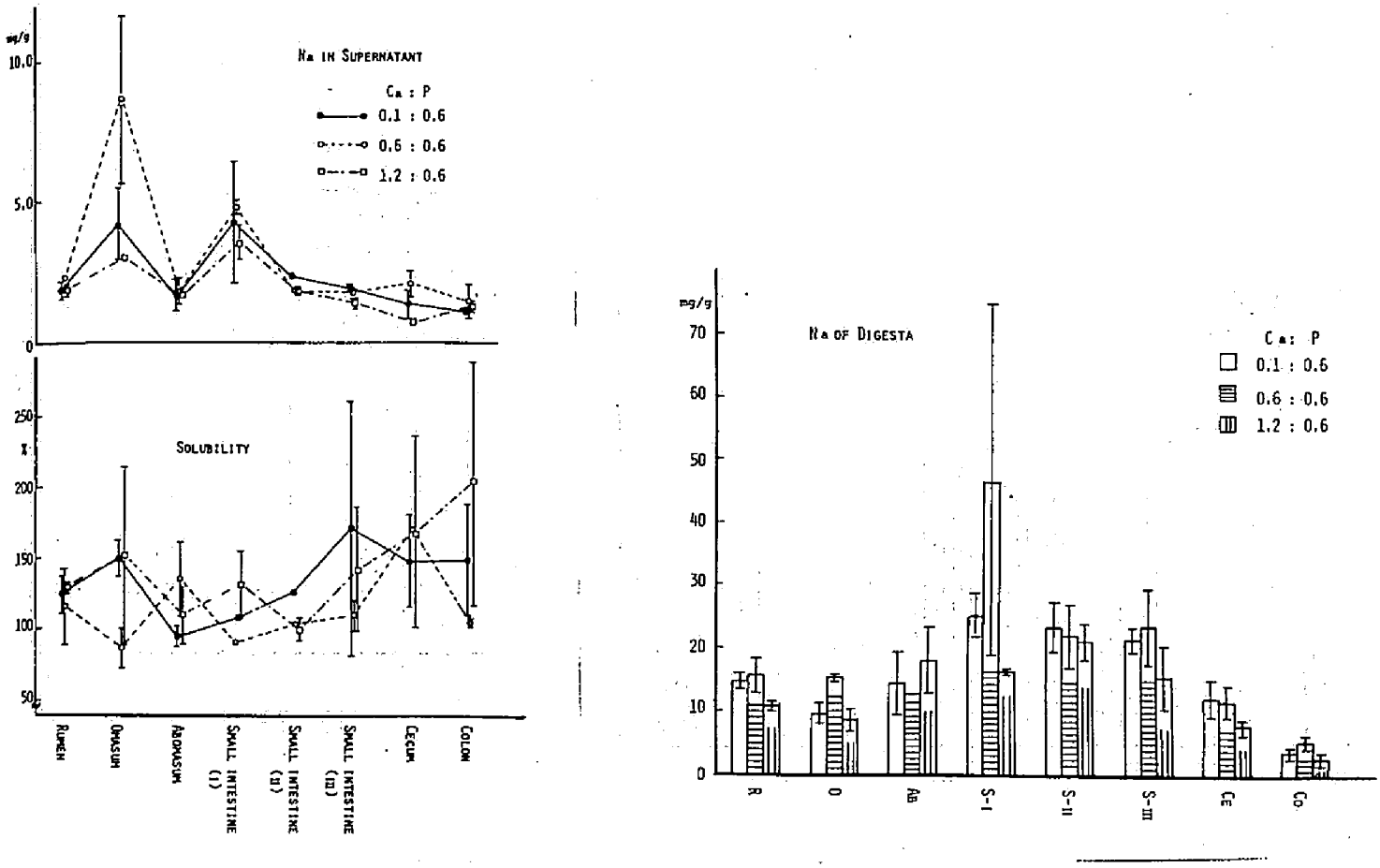


Fig. 5-4 Sodium concentration in supernatant and digesta, and sodium solubility at different sites of digestive tract

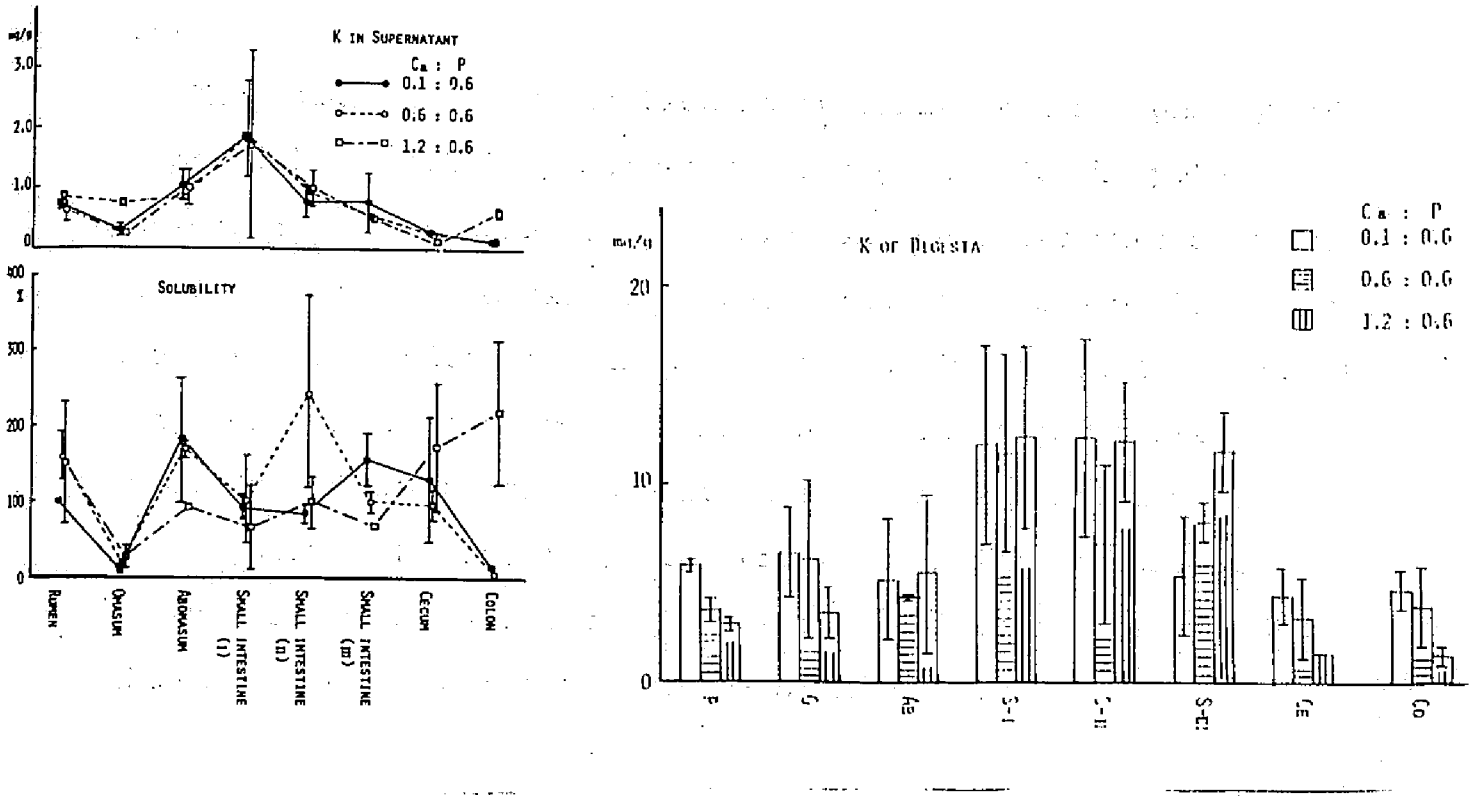


Fig. 5-5 Potassium concentration in supernatant and digesta, and potassium solubility at different sites of digestive tract

The sodium content in digesta appeared to be lower in the heighest calcium ration than in the other two rations in the sites of rumen, the lower part of small intestine, cecum and colon.

The potassium concentration in supernatant and digesta, and the potassium solubility are shown in figure 5-5. The potassium concentration in supernatant was high in the upper part of small intestine and then decreased in the following sites of digestive tract. The potassium solubility was obviously decreased in omasum. Among three treatment groups, there appeared to be no clear difference in the potassium concentration in supernatant and in the potassium solubility. The total potassium content in digesta tended to be lowered in the sites of rumen, cecum and colon as increasing the dietary calcium level.

Discussion

The calcium concentration in supernatant was lower in the lowest calcium ration than in the other two rations. The changes of calcium concentration in supernatant in two high calcium rations were parallel with the changes of the calcium solubility. The calcium concentration in supernatant and the calcium solubility seemed to be heightened as decreasing the pH value of digesta when the ration contained 0.6 or 1.2 % calcium. Therefore, the change of calcium concentration in supernatant and the calcium solubility would be strongly affected by the pH value of digesta.

Storry[83] reported that the proportion of ultrafiltrable calcium in the small intestine appeared to be dependent on the pH of the digesta, and low pH values favouring higher proportions of ultrafiltrable calcium.

On the other hand, the calcium in supernatant and the calcium solubility were not correlated with the change of the pH value of digesta when the dietary calcium level was 0.1 %. The calcium concentration in supernatant and the calcium solubility would not have the relationship to the pH value of digesta when a dietary calcium level was extremely low.

The decrease of calcium concentration in supernatant was found in the upper and the middle part of small intestine. Although the decrease of calcium concentration in supernatant would be partly owing to the decrease of solubility, the decrease would be partly due to the absorption. Therefore, the sites of calcium absorption found in this experiment was in accordance with the report of Perry et al.[66] that calcium had been almost absorbed in the intestine of calves.

The phosphorus concentration was high in omasum and the upper part of small intestine. The phosphorus solubility was also high in omasum, but was not so high in the upper part of small intestine. The former increase may be resulted from the increase of solubility and the latter increase may be due to phosphorus secretion. Smith et al.[79] and Chandler et al.[12] reported that the upper part of small intestine was the main part of phosphorus secretion in calves.

The phosphorus concentration in supernatant and the phosphorus solubility appeared to be lowered in the lower part of small intestine, cecum and colon as increasing the dietary calcium level. It was considered that the phosphorus concentration in supernatant was decreased by the formation of unsoluble calcium phosphate complex when the high dietary calcium ration was given. This result was in accordance with the assumption of Gill et al.[31] that most of the food phosphate was converted in the gut to the non-absorbable calcium phosphate. The total phosphorus content in digesta tended to be increased in the sites of the middle and the lower part of small intestine, cecum and colon when the dietary calcium level increased. This result would be due to that unabsorbable calcium phosphate complex was formed in the intestine and the absorption of phosphorus was decreased with the addition of calcium carbonate to the ration.

An evident difference was not found in sodium and potassium concentrations in supernatant among treatment groups. However, the total sodium and potassium contents in digesta were lower in the sites of cecum and colon in high dietary calcium ration than in low dietary calcium ration. The solubility of sodium and potassium in digesta were higher than those of calcium, phosphorus and magnesium. Therefore, this result would be induced by high solubilities of sodium and potassium.

It was reported by some workers[32,67,81] that sodium and potassium were effectively absorbed in large intestine. The lower contents of sodium and potassium in the sites of cecum and colon would be

resulted from the absorption of these minerals.

Sodium and potassium contents in rumen digesta were also decreased as increasing the dietary calcium level. This may be owing to the decrease in sodium and potassium excretion through saliva or the increase in absorption of these minerals through rumen. Yang and Thomas[104] indicated the absorption of sodium and potassium from rumen wall.

The magnesium, sodium and potassium concentrations in supernatant were increased in the upper part of small intestine. Ben-Ghedalia et al.[6] and Perry et al.[80] suggested the magnesium secretion in the upper part of small intestine. Perry et al.[80] also reported that sodium and potassium were excreted in the upper part of small intestine. The result found in this experiment supported these observations.

Summary

This experiment was conducted to study the effect of dietary calcium level on the concentration and the solubility of minerals in the digestive tract.

Six wethers were divided into three groups, two wethers each,. The each group was given the rations which was adjusted to 0.1, 0.6 and 1.2 % of calcium contents by adding calcium carbonate and 0.6 % of phosphorus by adding disodium phosphate to the ration. After sixtyseven days feeding, all sheep were slaughtered at two hours after the morning feed. Digesta samples were collected from rumen, omasum, abomasum, upper small intestine, middle small intestine, lower small intestine, cecum and colon. Polyethylene Glycol 4000 (PEG) was utilized as a marker and the concentrations of supernatant was showed as corrected values with this marker.

The calcium concentration in supernatant and the calcium solubility appeared to be heightened as decreasing the pH value of digesta when the ration contained 0.6 and 1.2 % calcium. The phosphorus concentration in supernatant and the phosphorus solubility were lowered and the total phosphorus content of digesta was increased in the middle and the lower part of small intestine, cecum and colon as increasing the dietary calcium level. This result suggests that unsoluble calcium phosphate complex was formed in the gut and the absorption of phosphorus was interfered by giving a high dietary calcium ration.

The total sodium and potassium concentrations of digesta

were lowered in cecum and colon which high dietary calcium rations were given. The decrease of sodium and potassium contents of digesta may be resulted from the increase of the absorption of these minerals.

CHAPTER 6 EFFECT OF VITAMIN A DEFICIENCY ON MINERAL METABOLISM AND INCIDENCE OF URINARY CALCULI

It was reported that vitamin A deficiency might occur in fattening cattle when high-concentrate and low roughage rations were given[94]. Schmidt[75] reported that goats given a low vitamin A ration developed urolithiasis. However, Beeson et al.[5] and Lindley et al.[57] indicated that the occurrence of urolithiasis did not increase when sheep were given a vitamin A deficient ration. Swingle and Marsh[86] and Elam et al.[21] also found no relationship between vitamin A deficiency and the occurrence of urolithiasis in cattle and sheep.

The present experiment was conducted to find the relationship between vitamin A deficiency and the occurrence of urolithiasis by studying the effects of vitamin A deficiency on urine and serum mineral concentrations and the morbid changes of urinary organ.

Materials and Methods

Trial 1 : Six wethers, averaging about 35 kg, were given a low vitamin A ration for approximately five months from summer to winter. After then, a level of 2000 I.U. of vitamin A per head was supplemented to the ration daily during successive one month. Urine and serum samples were obtained during the last week of the fifth month after the beginning of the experiment,

low vitamin A period, and the last week of vitamin A supplemented, sixth month,. The ration was given twice daily and water was available at all times. The composition of basal ration and its mineral content are shown in table 6-1 and 6-2.

Table 6-1 Composition of low vitamin A rations

Ingredients	Trial	
	1	2
Barley	20 %	48 %
Ground corn	36	-
Wheat bran	12	12
Rice bran	5.6	16
Soybean meal	4.8	4
Rice straw	20	20
Sodium chloride	0.8	-
Calcium carbonate	0.8	-

Table 6-2 Mineral composition of the basal rations

(% of air dry matter)

Mineral	Trial	
	1	2
Calcium	0.38	0.20
Phosphorus	0.52	0.85
Magnesium	0.17	0.27
Sodium	0.38	0.30
Potassium	0.64	0.84

Urine samples were obtained at ten o'clock every morning from each wethers. After determining the pH values, a few drops of acetic acid and toluen were added to the urine samples and stored in the stocker at -20 C for analysis. Blood samples were obtained by jugular vein puncture and stored for analysis. Serum and liver vitamin A values were determined by the modified method of the Association of Vitamin Chemists [3] . The analytical methods of mineral concentration in urine and serum were the same as those in chapter 4.

Trial 2 : Four wethers, averaging about 31 kg, were alloted to two groups two wethers each. The low vitamin A ration shown in table 6-1 was given to all wethers for one year. The supplement of 3000 I.U. of vitamin A and 200 I.U. of vitamin D per head a day were given to one group (vitamin A supplemented) and were not given to another group (low vitamin A). Urine and serum samples were obtained in the last week of one year experimental period. Sampling and analytical procedure were similar to those used in trial 1. After collecting the urine and the serum samples, all wethers were slaughtered and liver samples were obtained in order to determine vitamin A levels of the livers. The tissue samples from the kidney, the bladder and the urethra were collected from every wethers and these samples were placed in formalin, embedded in paraffin and stained in two ways, with hematoxylin and eosin and with hematoxylin and PAS.

Results

Trial 1 : The levels of plasma vitamin A throughout the experiment are shown in table 6-3. The plasma vitamin A level at the end of low vitamin A period was decreased in one third when compared to that of the beginning. The plasma vitamin A level in the vitamin A supplemented period went back to the initial level. The data of urine and serum mineral levels in trial 1 are shown in table 6-4.

There appeared to be no obvious differences in the urine and serum mineral levels between the low vitamin A period and the vitamin A supplemented period. Though the urine volume tended to be larger and the urine pH to be lower in the low vitamin A period than in the vitamin A supplemented period. However, these differences between both periods were not significant.

Trial 2 : The result of this trial is shown in table 6-5 and 6-6. The liver vitamin A level of the low vitamin A group was decreased about one twentieth of that of the supplemented group. Particularly, that of wether No. 4 was so low that this animal seemed to be led to vitamin A deficiency. Urine and serum mineral levels of wether No. 3 in the low vitamin A group were not different from those in the vitamin A supplemented group.

However, the urine volume of wether No. 4 appeared to be 25 % to 50 % less and urine phosphorus, potassium and ammonia levels of the same animal were apparently higher than those of

Table 6-3 Plasma vitamin A level in wethers (Trial 1)

Period	Plasma vitamin A (mcg/100 ml)
Beginning of trial	98 ± 14
End of low vitamin A period	32 ± 4
End of vitamin A supplemented period	84 ± 14

Table 6-4 The effect of low vitamin A on urine and serum mineral levels in wethers (Trial 1)

	Vitamin A supplemented period	Low vitamin A period
Urine values, mg/100 ml		
Calcium	0.78	0.86
Phosphorus	78.1	71.3
Magnesium	30.9	26.1
Sodium	245	235
Potassium	414	453
Chloride	369	423
Urine volume, ml	814	1114
Urine pH	8.72	7.76
Serum values, mg/100 ml		
Calcium	8.37	8.61
Phosphorus	10.0	11.1
Magnesium	3.28	3.03
Sodium	339	346
Potassium	20.6	24.1

Table 6-5 Effect of vitamin A deficiency on urine and serum mineral levels (Trial 2)

Group	Vitamin A supplemented		Low vitamin A	
Wether	No.1	No.2	No.3	No.4
Liver vitamin A, mcg/g	53.7	24.3	3.7	0.5
Urine values, mg/100 ml				
Calcium	1.16	0.80	0.32	0.45
Phosphorus	47.6	23.8	35.0	193.3
Magnesium	16.3	12.7	13.0	31.6
Sodium	14.0	6.6	14.2	12.9
Potassium	182	120	155	714
Chloride	117	154	198	132
Ammonia	141	96	89	693
Urine volume, ml	1369	2354	2619	675
Urine pH	8.53	8.27	8.64	8.79
Plasma values, mg/100 ml				
Phosphorus	11.5	9.8	8.2	13.4
Magnesium	3.1	4.3	4.8	3.3
Sodium	344	354	350	369

Table 6-6 Effect of vitamin A deficiency on urine mineral excretion (Trial 2)

(mg/day)

Group	Vitamin A supplemented		Low vitamin A	
Wether	No.1	No.2	No.3	No.4
Calcium	15	20	9	3
Phosphorus	646	451	936	1262
Magnesium	216	305	374	179
Sodium	199	192	351	78
Potassium	2545	2689	4089	4733
Chloride	1362	3285	4667	908
Ammonia	1984	2014	2348	4950

other wethers. The urine magnesium and the serum phosphorus of the same animal appeared to be somewhat higher than those of other wethers. The results of microscopic examination of the kidney, the bladder and the urethra are shown in table 6-7 and figures.

Table 6-7 Characteristics of renal morbid changes

Morbid changes	Group of wethers	
	Vitamin A supplemented	Low vitamin A
Renal pelvis		
Desquamated epithelium cells	-	+
Renal tubule		
Desquamated epithelium cells	+	++
Disappearance of nuclei	-	++
Atrophy	-	+
Necrosis	-	+
Polysaccharide urinary casts	+	++
Granular protein substance in glomeruli	-	++

The symbols, -, +, ++, +++, show the severity of lesions (-: normal, +: mild, ++: moderate, +++: severe).

At the autopsy, there appeared to be no difference in weight, shape and color of the kidneys between the low vitamin A group and the vitamin A supplemented group. Urinary calculi were not found visually in the urinary organs of all wethers, while sand-like micro calculi were found in the pelvis of the kidney of wether No.4 by microscopic examination (fig. 6-1). Though PAS positive urinary casts in renal tubule were found in all wethers, the amount of them was higher in the low vitamin A group than in the vitamin A supplemented group.

Some morbid changes were observed in the renal tubule of the low vitamin A wethers. The desquamated renal epithelium including PAS positive materials were also found in the pelvis of the low vitamin A wethers (fig. 6-2 and 6-3). These renal morbid changes suggested the occurrence of nephrosis in the kidney. The separation of transitional epithelium was found in the bladder and the urethra of the low vitamin A wethers. On the other hand, the kidney, the bladder and the urethra of the vitamin A supplemented wethers were little affected (fig. 6-4 and 6-5).

Discussion

Swingle and Marsh[86] reported that the average liver vitamin A level in calves of the deficient group was 1.4 mcg per g and some of them had nightblindness. Elam et al.[21] also reported that the average liver vitamin A content in the deficient steers having nightblindness was 0.33 mcg per g. Recently, Kohlmeir et al.[49] reported that no dietary vitamin A was required for good feedlot performance as long as plasma and liver vitamin A levels remained above 25 mcg per 100 ml and 2 mcg per g, respectively.

The plasma vitamin A in the end of the low vitamin A period in trial 1 was 32 mcg per 100 ml. Therefore, the animals might not need the supplemental vitamin A to maintain normal function. This may be the reason why the obvious differences were not found in the urine and the serum mineral levels between the low vitamin A period and the control period. Liver vitamin A level of the wether No. 3 and No. 4 of low vitamin A group in trial 2 was 3.7 and 0.5 mcg per g, respectively. Liver vitamin A level of wether No. 3 seemed to be low but not below the critical level, while that of wether No. 4 might be below the critical level of vitamin A deficiency.

In this experiment, the urine volume of wether No. 4 was less than those of the other wethers. Woelfel et al.[101] reported that the urine volume was increased in vitamin A deficient heifers and Webb et al.[98] reported that the similar result in

ewes. However, Webb et al.[99] indicated in the following report that glomerular filtration rate and renal plasma flow were significantly decreased in vitamin A deficient ewes.

The increase of the urine phosphorus level was found in the wether No. 4 in the low vitamin A group. This observation was in agreement with the report of Woelfel et al.[101] and Webb et al.[98,99]. As previously mentioned in chapter 3, the increase of the urine phosphorus level may cause the formation of urinary calculi in ruminants.

Urine magnesium and potassium levels appeared to be higher in wether No. 4 than those in other wethers. While, daily excretion of urine magnesium and potassium in wether No. 4 was not different from those in the others. Therefore, the increase of urine magnesium and potassium levels may be caused by the decrease of the urine volume in the vitamin A deficient wether.

There seemed to be no difference in urine calcium, sodium and chloride concentrations between wether No. 4 and the other wethers. However, daily excretion of urine calcium, sodium and chloride were lower in wether No. 4 than those in the others. This result was in agreement with the results of Webb et al.[98].

Urine ammonia level and daily excretion of urine ammonia were evidently increased in the vitamin A deficient wether. As previously mentioned (chapter 2), urinary calculi which were found in the prepuital hair seemed to be made of magnesium ammonium phosphate. Therefore, it was suggested that the

formation of urinary calculi in the prepuce and the prepuital hair might be accelerated with the increase of urine ammonia level. But the effect of urine ammonia level on calculi formation in the kidney, the bladder and the urethra was not known clearly.

Plasma phosphorus level was found to be higher in the wether No. 4 than those of the others. Webb et al.[98] also reported that plasma phosphorus level was increased in vitamin A deficient wethers. However, Swingle and Marsh[86] reported that there was no difference in plasma phosphorus level between vitamin A dificient calves and the control. The different result of these studies may be due to the degree of vitamin A deficiency. Liver vitamin A level was 0.5 mcg per g in this experiment and 1.4 mcg per g in the study of Swingle and Marsh[86]. Some workers[9,16,24] indicated that the increased serum phosphorus level may be associated with the occurrence of urolithiasis.

It was found in the low vitamin A wethers that the kidney had nephrosis and the epithelium of the bladder and the urethra were also affected to some extent. Micro calculi were found in the kidney of the vitamin A deficient wether. Higgins et al.[37] reported that the incidence of keratinization of the epithelium in the urinary tract and urolithiasis were increased in vitamin A deficient rats. Schmidt[75] reported that a large masses of desquamated materials, and cast-off cornified epithelium cells were found in the pelvis of the kidney, and the occurrence of urolithiasis were increased in vitamin A deficient goats.

Prien[70] discussed in his review that blood clot and desquamated epithelium might become the nuclei and accelerate the formation of urinary calculi. However, some workers[8,16,24] reported that there was no relationship between vitamin A deficiency and the occurrence of urolithiasis in sheep and cattle.

Since the urine and serum mineral levels and histological changes of urinary organs may be caused by only the serious vitamin A deficiency, it may be safe to consider that vitamin A dificiency becomes a causative factor of urinary calculi, but is not a essential one.

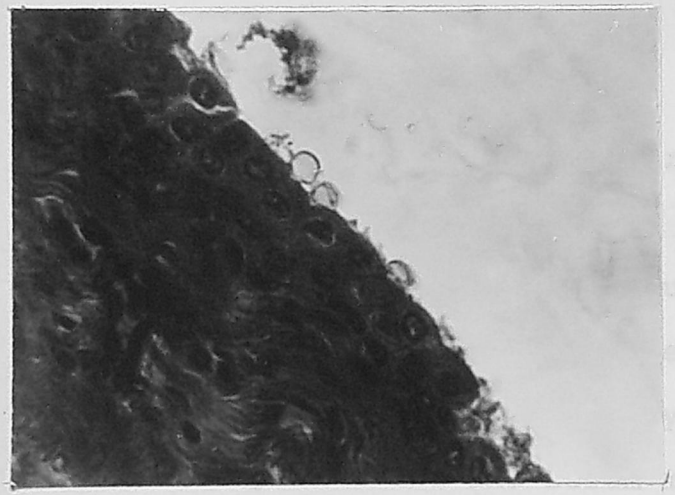


Fig. 6-1 Sand-like micro calculi were found in the pelvis of the kidney of the vitamin A deficient wether, x 400, HE staining.

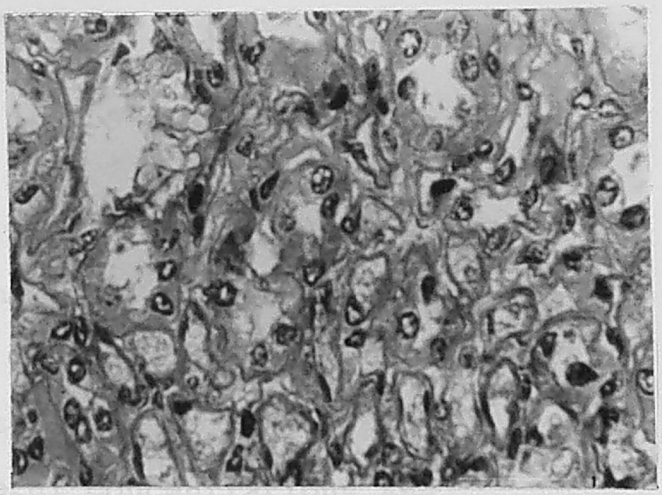


Fig. 6-2 Disappearance of nuclei epithelium cells and desquamated renal epithelium cells in the low vitamin A wether, x 200, HP staining.

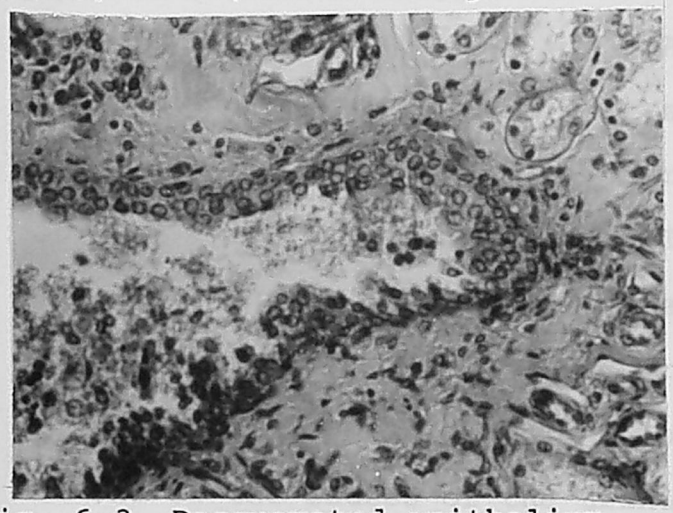


Fig. 6-3 Desquamated epithelium cells in renal pelvis of the low vitamin A wether, x 200, HP staining

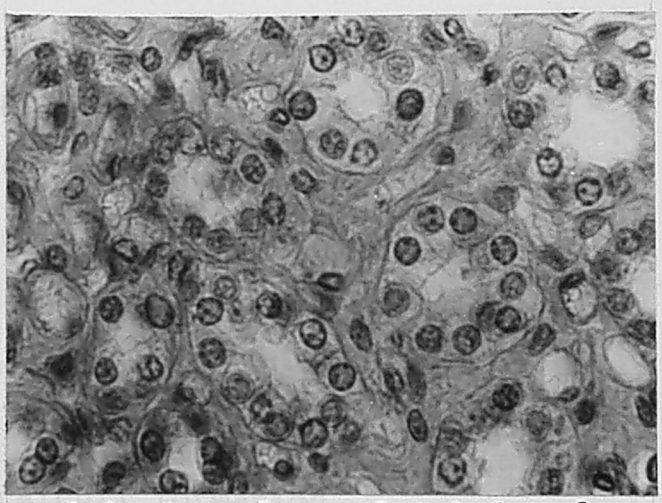


Fig. 6-4 Normal renal tubule of the vitamin A supplemented wether, x 400, HE staining.

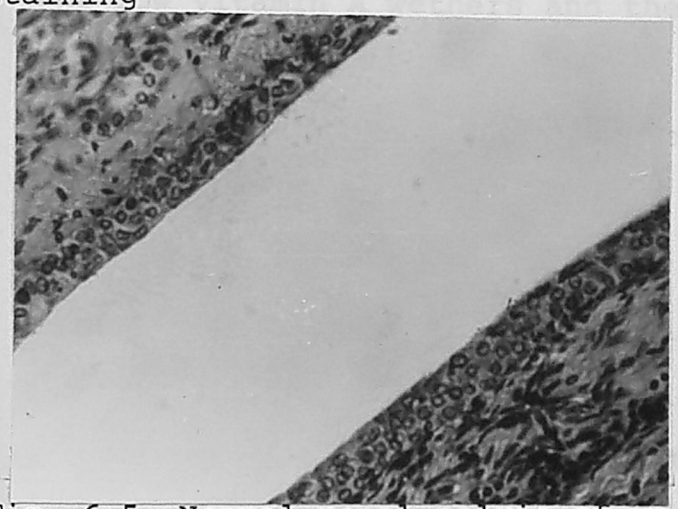


Fig. 6-5 Normal renal pelvis of the vitamin A supplemented wether, x 200, HP staining.

Summary

The present experiment including ten wethers was conducted to examine the relationship between vitamin A deficiency and the occurrence of urolithiasis by studying the effect of vitamin A deficiency on urine and serum mineral concentrations and the morbid changes of urinary organ.

Micro-calculi were found by microscopic examination in the renal pelvis of the vitamin A deficient wether, but not in vitamin A supplemented wethers. The urine volume of the vitamin A deficient wether appeared to be smaller than the vitamin A supplemented wethers. The amount of urine phosphorus, potassium and ammonium and the serum phosphorus of the vitamin A deficient animal seemed to increase. It is suggested that the increase of the urine phosphorus and ammonium levels may be one of the causes of the urinary calculi formation.

The occurrence of nephrosis was found in the kidney of the low vitamin A wethers and these animals had some amount of the desquamated epithelium of the bladder and the urethra. The desquamated epithelium may become the nuclei of urinary calculi.

However, the changes of the urine and serum mineral concentrations, and the histological abnormalities of urinary organs were found only among serious vitamin A deficient animals. Therefore, vitamin A deficiency may become a causative factor of urinary calculi, but may not be a essential one.

CAHPTER 7 EFFECT OF ADDITION OF AMMONIUM CHLORIDE ON MINERAL METABOISM AND INCIDENCE OF URINARY CALCULI

SECTION 1 Effect of Ammonium Chloride Administration on Urine and Serum Mineral Levels in Wethers

Several dietary supplements have been shown to reduce the incidence of urinary calculi in sheep and cattle[10,11,27,89,90]. Ammonium chloride seemed to be most effective against urinary calculi among these supplements[11,15]. Uesaka et al.[93] reported that ammonium chloride was effective in the treatment and the prevention of urolithiasis in fattening steers in Japan. However, the mechanism of ammonium chloride against urolithiasis has not been clarified yet. If the cause why ammonium chloride is effective may be known, the inducing factor of urinary calculi may be demonstrable.

There are two experiments in this chapter, that is, in vivo and in vitro. Firstly, the changes of urine and serum mineral composition were examined when ammonium chloride was given to wethers. And then, the mechanism of ammonium chloride was studied by preparing the similar changes in vitro which was found in urine composition.

The object of the experiment in section 1 was to determine the effect of ammonium chloride on the urine and serum mineral levels and mineral retention.

Materials and Methods

This experiment consisted to four trials. The effect of ammonium chloride in the urine and serum mineral levels was examined in three trials and the fourth was the mineral balance trial. The composition of basal ration and its mineral content are shown in table 7-1 and 7-2.

The ration was given twice daily and water was provided ad libitum. Ammonium chloride was given at the level of 1 % of the ration. Sampling procedure and the method of mineral analysis were the same as those in chapter 4.

Trial 1 : Four wethers about 30 kg in weight were fed for 70 days in winter. After feeding for two months, all wethers were given the ration supplemented with ammonium chloride for two weeks. Urine and serum samples were obtained at one week before and after ammonium chloride was given.

Trial 2 : Six wethers, averaging about 37 kg, were fed for six weeks in early spring. All wethers were given ammonium chloride during the third and the fourth week. Urine and serum samples were obtained during the fourth week, and during the second and the fifth week as controls.

Trial 3 : Four wethers, averaging approximately 31 kg, were divided into two groups, two wethers each and were fed for four weeks in summer. The one group was administered ammonium chloride and the other group was left for the control. Urine and serum samples were obtained during the fourth week.

Table 7-1 Composition of the basal rations

Ingredients	Trial		
	1	2	3 and 4
Barley	20 %	20 %	48 %
Ground corn	45	45	-
Wheat bran	12	12	12
Rice bran	6	6	16
Soybean meal	5	5	4
Rice straw	20	20	20
Sodium chloride	1	1	-
Calcium carbonate	1	1	-

Table 7-2 Mineral composition of the basal rations

Minerals (% of dry matter)	Trial		
	1	2	3 and 4
Calcium	0.34	0.38	0.20
Phosphorus	0.51	0.52	0.85
Magnesium	0.17	0.17	0.27
Sodium	0.51	0.38	0.30
Potassium	0.85	0.64	0.84

Trial 4 : The effect of ammonium chloride on mineral retention was examined. The same wethers, rations and experimental design were used as trial 3. After preliminary ten days from the beginning of the trial, urine and fecal samples were obtained for seven days.

Results

The symptoms of urolithiasis was noticed in two wethers at the first sampling in trial 1 and these wethers were given ammonium chloride at the level of 1 % of the ration daily until the symptoms of urolithiasis disappeared. Therefore, the second sampling was delayed about one month. In trial 2, 3 and 4, bloody urine was observed, but urolithiasis did not occur.

The results of urine mineral concentrations, urine pH values and urine volumes are shown in table 7-3. In three trials, the administration of ammonium chloride increased the urine calcium and chloride levels and decreased the urine pH. The urine phosphorus level was slightly increased after the administration of ammonium chloride.

There were some differences in the urine mineral concentrations and the urine volume among three trials. The urine calcium level was lower and the urine phosphorus level was higher in trial 1 and 3 than those in trial 2. The sodium and chloride levels of urine were apparently lower in trial 3 than in trial 1 and 2.

Serum data from trial 1,2 and 3 are shown in table 7-4.

Table 7-3 Effect of ammonium chloride administration on urine volume, pH values and mineral levels

Trial	1		2		3	
Treatment	Control	+NH_4Cl	Control	+NH_4Cl	Control	+NH_4Cl
Urine values, mg per 100 ml						
Calcium	0.8	32.3	4.3	36.5	0.4	9.0
Phosphorus	140	213	27	29	120	132
Magnesium	61	74	67	41	26	28
Sodium	382	276	244	252	1.3	13.3
Potassium	610	594	564	378	323	298
Chloride	577	953	522	983	139	274
Urine volume, ml	651	794	817	1003	2690	3451
Urine pH	6.95	5.52	8.17	6.85	7.16	6.36

Table 7-4 Effects of ammonium chloride on serum mineral levels in wethers

Trial	1		2		3	
Treatment	Control	+NH_4Cl	Control	+NH_4Cl	Control	+NH_4Cl
Serum values, mg per 100 ml						
Calcium	6.7	6.2	11.0	11.4	-	-
Phosphorus	10.7	10.2	7.2	6.7	11.0	11.0
Magnesium	3.4	3.3	3.3	2.9	4.7	2.9
Sodium	388	347	400	346	358	364
Potassium	21	20	19	21	-	-

Ammonium chloride administration appeared not to affect the serum mineral levels except magnesium. When compared with the control, the serum magnesium level tended to decrease slightly in wethers given ammonium chloride. The serum calcium level appeared to be lower and the serum phosphorus level to be higher in trial 1 than those in trial 2.

The result of the mineral balance trial, trial 4, are shown in table 7-5. The urine calcium excretion was considerably higher in wethers administered ammonium chloride than those in the control wethers. But the calcium excretion in the urine was much lower than that of fecal excretion. And the calcium retention appeared to be the same between the control and the treatment animals. The excretion of urine phosphorus appeared to be slightly higher and the fecal excretion of phosphorus to be lower in the treatment group than in the control. The fecal sodium excretion in wethers given ammonium chloride appeared to be decreased to one half of the control. The sodium retention appeared to be increased in the treatment group. Ammonium chloride administration appeared not to affect magnesium and potassium retentions.

Table 7-5 Effect of ammonium chloride administration on mineral balances

(g per day)

	Control			+NH_4Cl		
Wether No.	1	2	Mean	3	4	Mean
Calcium						
Intake	3.17	3.58	3.38	3.38	3.46	3.42
Urinary excretion	0.009	0.003	0.006	0.129	0.068	0.099
Fecal excretion	2.51	1.84	2.17	2.06	2.20	2.13
Retention	0.65	1.74	1.20	1.19	1.19	1.19
Phosphorus						
Intake	6.94	7.84	7.39	7.38	7.57	7.48
Urinary excretion	0.94	1.26	1.10	2.45	0.78	1.62
Fecal excretion	2.94	2.55	2.74	1.30	2.86	2.08
Retention	3.07	4.03	3.55	3.64	3.93	3.79
Magnesium						
Intake	2.19	2.47	2.33	2.33	2.39	2.36
Urinary excretion	0.37	0.18	0.28	0.28	0.32	0.30
Fecal excretion	1.11	1.18	1.15	1.26	0.96	1.11
Retention	0.71	1.11	0.91	0.79	1.10	0.95
Sodium						
Intake	2.49	2.81	2.65	2.65	2.72	2.68
Urinary excretion	0.35	0.08	0.22	0.34	0.12	0.23
Fecal excretion	0.52	0.44	0.48	0.12	0.30	0.21
Retention	1.62	2.29	1.96	2.18	2.30	2.24
Potassium						
Intake	6.90	7.80	7.35	7.34	7.53	7.43
Urinary excretion	4.09	4.73	4.41	5.88	3.43	4.66
Fecal excretion	1.33	1.31	1.32	0.32	1.93	1.13
Retention	1.48	1.76	1.62	1.14	2.17	1.65

Discussion

It was found in this experiment that urine pH values were lowered by the administration of ammonium chloride. Elam et al.[21] reported that magnesium and phosphorus, the principal constituents found in calculi, were insoluble in alkaline solution and the effect of decreasing urine alkalinity may prevent the precipitation of these minerals. Johnson[46], Elliot et al.[23] and Suby[84] reported that the solubility of magnesium ammonium phosphate increased when the concentration of hydrogen ion increased. On the other hand, Lindley et al.[57] showed that there were no apparent association between the occurrence of urolithiasis and the difference of the urine pH. Udall[90] also indicated that the urine pH was decreased by feeding phosphoric acid, but no corresponding difference was found in the occurrence of renal calculi. As previously mentioned (chapter 3), the urine pH may have little effect on the formation of urinary calculi. However, it is conceivable that the main function of ammonium chloride is to decrease the urine pH, which prevents the formation of urinary calculi.

It was found in this experiment that ammonium chloride increased the urine calcium excretion, while the amount of the fecal calcium excretion and retention were little affected. Bushman et al.[10] suggested that the increased urine calcium level may not affect the reduction of urinary calculi, and the decrease of urine pH may increase the urine calcium level.

It was found in this experiment that urine chloride level was increased by giving ammonium chloride. Udall and Chow[91] reported that the administration of sodium chloride reduced the occurrence of urolithiasis due to the increased urine chloride ion which caused ion competition around calculi. Bushman et al.[11] postulated that the increased urine chloride level did not protect against calculi formation.

The serum magnesium level decreased in wethers when ammonium chloride was given. Bushman et al.[11] also reported the similar result. Packett and Haushild[64], Crookshank et al.[16] and Kunkel et al.[51] reported that high serum magnesium levels were found in sheep developed urolithiasis. However, it was not known clearly whether the decrease of the serum magnesium level has a direct effect to the reduction of urolithiasis or not.

The urine volume appeared to be increased a little by feeding ammonium chloride. Since ammonium chloride has been used as a dieuretic, the increase of urine volume may be natural. It was considered that the increase of urine volume would lead to the dilution of urine minerals and other urine components, and interfere the formation of urinary calculi. This was supported by the work of Newsom et al.[62], urine volume was low in sheep which developed urolithiasis. However, the prevention on calculi formation by increasing the urine volume may be limited, because the increase of urine volume is not so large. Swingle and Marsh[85] and Udall et al.[92] reported that the increase of urine volume had no effect on reducing the occurrence of urolithiasis.

Bushman et al.[10,11] showed that there was no increase of the urine sodium level by giving ammonium chloride. In this experiment, the increase of urine sodium level was found in wethers given ammonium chloride in trial 3, while any change in urine sodium was not found in the treated wethers in trial 1 and 2. The urine sodium level was remarkably lower in trial 3 than in trial 1 and 2. This result may be due to that sodium chloride was not supplemented in trial 3. It was considered that the administration of ammonium chloride might affect the urine sodium level, but its effect might depend on the amount of sodium present in the body. As previously mentioned (chapter 3), the occurrence of urolithiasis was associated with the high urine sodium level. Therefore, the increase of urine sodium level seemed not to reduce the occurrence of urolithiasis. This assumption was supported by the report of Udall and Chow[91] and Bushman et al.[11] that there appeared to be no relationship between urine sodium level and the occurrence of urolithiasis.

When compared with the control, the fecal sodium excretion was decreased and the sodium retention was increased in wethers given ammonium chloride. While, Bushman et al.[11] showed that there were no differences between sheep given ammonium chloride and the control animals in the fecal sodium excretion and the sodium retention. The contradiction between two studies might be due to the different degree of the urine pH depression caused by the administration of ammonium chloride. By giving ammonium chloride, the urine pH was depressed about 0.8 in this experiment,

and about 0.38 in the report of Bushman et al.[11]. The effect of the administration of ammonium chloride on the fecal sodium excretion and the sodium retention seemed to be related to the acid-base balance in an animal body.

Summary

Four trials, utilized eighteen wethers, were conducted to determine the effect of ammonium chloride on the urine and serum mineral levels and mineral retentions. Ammonium chloride was given at the level of 1 % of the ration daily.

When compared with the control wethers, the urine volume of animals given ammonium chloride tended to increase slightly and the difference of the urine pH values between the control and the treated animals were 0.86 and 1.43.

The administration of ammonium chloride increased the urine calcium concentration 10 to 40 times, doubled the urine chloride concentration and decreased the serum magnesium level to some extent. When ammonium chloride was given to animals of which the urine sodium was low, the urine sodium level increased 10 times, the fecal sodium content tended to decrease, and sodium retention increased.

Among these effects of ammonium chloride, the reduction of the urine pH value seemed to be the main factor to decrease the incidence of urolithiasis.

SECTION 2 The Mechanism of Ammonium Chloride on the Prevention of Urolithiasis

The experiment in section 1 of this chapter showed that the addition of ammonium chloride to a ration had increased the urine volume and the urine concentrations of calcium, sodium and chloride, and decreased the urine pH value in wethers.

Though the increase of urine volume may be expected to prevent urolithiasis, the relation between the changes of urine components and the treatment of urinary calculi is not definitely known. Assuming the changes of urine components by giving ammonium chloride, these conditions were prepared in vitro.

This experiment was conducted to determine the mechanism of ammonium chloride through the changes of solubilities of magnesium phosphate and magnesium ammonium phosphate in in vitro condition.

Materials and Methods

In this experiment, the solubilities of magnesium phosphate ($MgHPO_4 \cdot 3H_2O$) and magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) were examined when pH values and the levels of calcium, sodium ,chloride and ammonium were changed in tris-maleate buffer solutions. Two grams of magnesium

phosphate or magnesium ammonium phosphate were added in 300 ml aliquots of buffer solution in a tightly stoppered flask. The flask was incubated at 39°C and was shaken on a mechanical shaker for 6 hours.

The pH values of buffer solution were changed approximately 5.5, 6.5, 7.5 and 11.5 through the changes of tris-maleate and sodium hydroxide ratio. Calcium concentrations in buffer silution were varied to 0, 10, 20, 30 and 40 mg% by the addtion of calcium chloride or calcium carbonate. Sodium concentrations in buffer solution were varied to 0, 200, 400, 600 and 800 mg% by the addition of sodium acetate or sodium sulfate. Chloride levels were varied to 0, 200, 400, 600 and 800 mg% by the addition of sodium chloride. Ammonium levels were varied to 0, 200, 400, 600 and 800 mg% by the addition of ammonium sulfate or ammonium chloride.

Except for the trial with pH changes, pH values in aliquots of buffer solution were adjusted approximately to 8.0 which was normal pH value in cattle[48]. Samples of 5 ml were taken from each flask at intervals of 0, 1, 2, 4 and 6 hours after the beginning of shaking. The pH values were determined in each sample soon after taking samples with a glass rod pH meter. Then these samples were centrifuged at 3000 rpm for 30 minutes, and the concentrations of phosphorus and magnesium in supernatant were determined by the method of Fiske and Subbarow[29] and by atomic absorption

spectrometer respectively.

Results

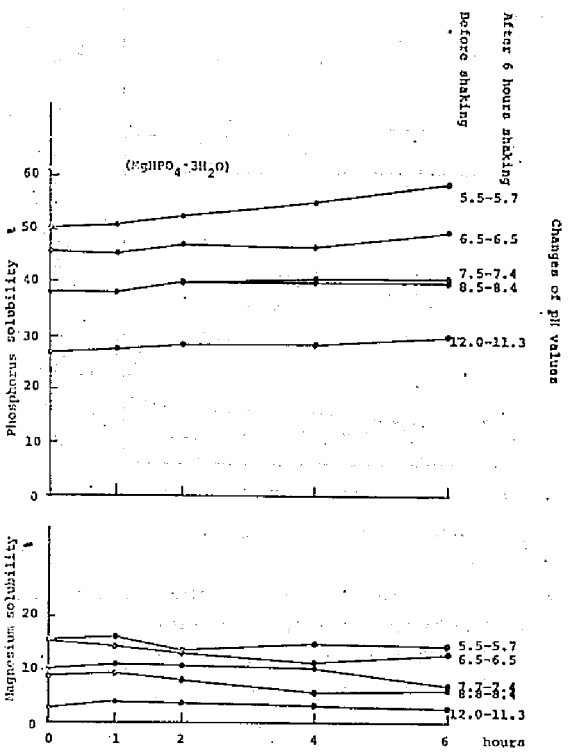


Fig 7-1-1 Effect of pH upon phosphorus and magnesium levels in buffer solutions after 6 hours shaking with $MgHPO_4 \cdot 3H_2O$.

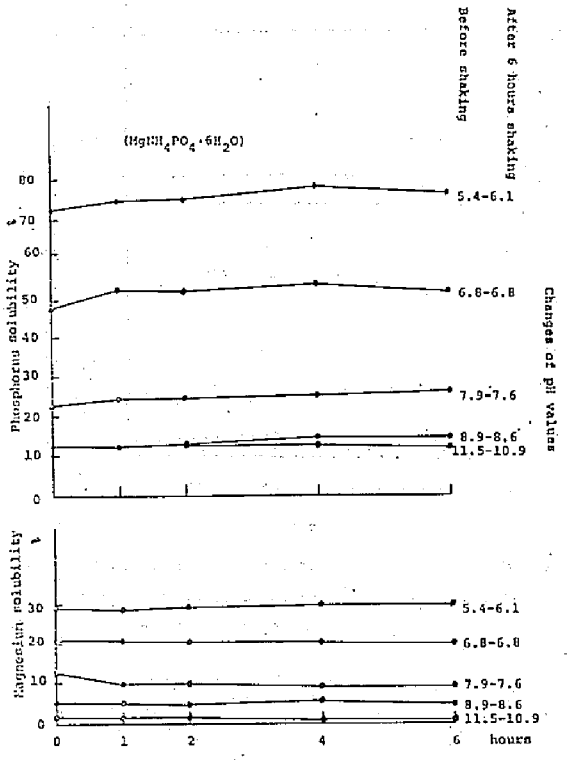


Fig 7-1-2 Effect of pH upon phosphorus and magnesium levels in buffer solutions after 6 hours shaking with $MgNH_4PO_4 \cdot 6H_2O$

As shown in figure 7-1-1 and 7-1-2, the solubilities of magnesium phosphate and magnesium ammonium phosphate were higher in buffer solutions of low pH than in solutions of

high pH. This trend was more obvious in magnesium ammonium phosphate than in magnesium phosphate.

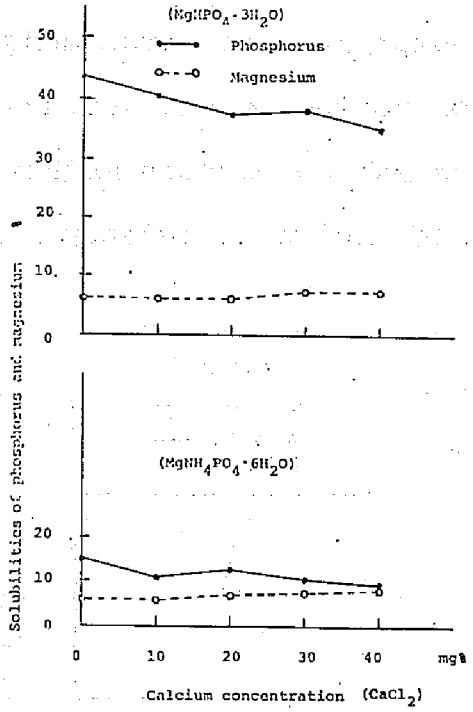


Fig 7-2 Effect of calcium concentration upon phosphorus and magnesium levels in buffer solutions after 6 hours shaking with $MgHPO_4 \cdot 3H_2O$ and $MgNH_4PO_4 \cdot 6H_2O$

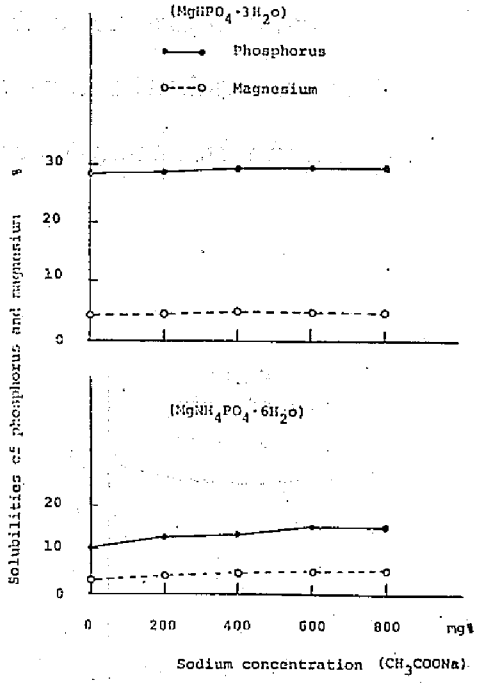


Fig 7-3 Effect of sodiun concentration upon phosphorus and magnesium levels in buffer solutions after 6 hours shaking with $MgHPO_4 \cdot 3H_2O$ and $MgNH_4PO_4 \cdot 6H_2O$.

As shown in figure 7-2, the phosphorus solubility after shaking with magnesium phosphate and magnesium ammonium phosphate tended to be decreased by increasing the calcium concentration. On the other hand, the magnesium solubility appeared to be increased with the elevation of calcium concentration after equilibration with magnesium phosphate. The result of this trial, which used calcium carbonate as calcium additive, was similar to that which used calcium chloride .

In figure 7-3, slight increases in phosphorus and magnesium solubilities were found by adding sodium acetate as sodium salts when equilibrated with magnesium ammonium phosphate. However, there was little change in the solubility of phosphorus and magnesium equilibrated with magnesium phosphate. The effect of sodium sulfate was similar to that of sodium acetate on the solubilities of magnesium phosphate and magnesium ammonium phosphate.

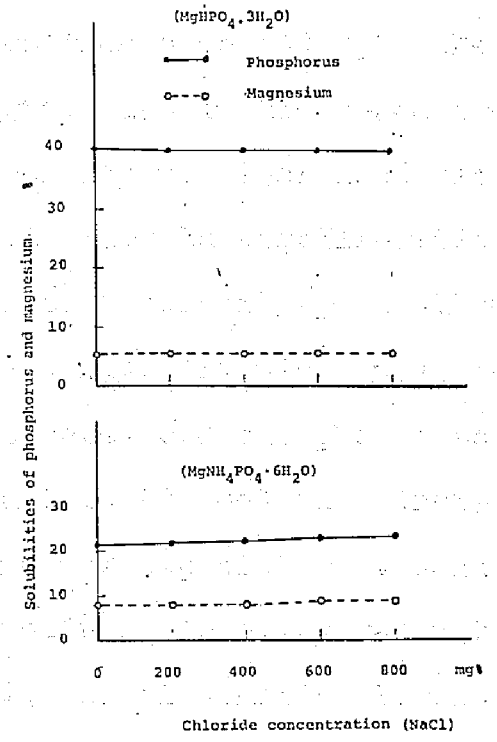


Fig.7-4 Effect of chloride concentration upon phosphorus and magnesium levels in buffer solutions after 6 hours shaking with $MgHPO_4 \cdot 3H_2O$ and $MgNH_4PO_4 \cdot 6H_2O$.

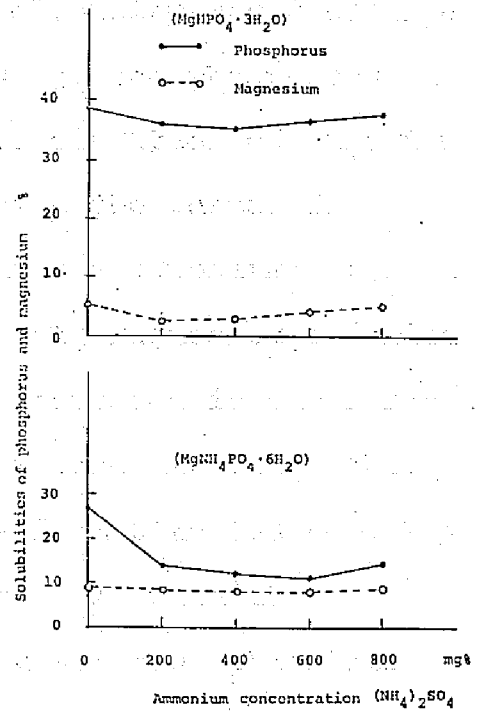


Fig.7-5 Effect of ammonium concentration upon phosphorus and magnesium levels in buffer solutions after 6 hours shaking with $MgHPO_4 \cdot 3H_2O$ and $MgNH_4PO_4 \cdot 6H_2O$.

As shown in figure 7-4, the increasing of chloride concentration caused only slight increases in phosphorus and magnesium solubilities after equilibration with magnesium ammonium phosphate. And noticeable changes were not found in phosphorus and magnesium solubilities by increasing the chloride concentration in buffer solutions when equilibrated with magnesium phosphate.

The results obtained by the addition of ammonium salts to aliquots of buffer solution were shown in figure 7-5. In the range from 0 to 400 mg%, the phosphorus solubility was decreased slightly and in the range from 400 to 800 mg% the phosphorus solubility was increased slightly with the elevation of ammonium level after the equilibration with magnesium phosphate. The magnesium solubility was also decreased at the ammonium level of 400 mg%. Likewise, when equilibration with magnesium ammonium phosphate, the phosphorus solubility was decreased in the range from 0 to 600 mg% with increasing ammonium concentration; this trend was especially evident in the range from 0 to 200 mg%. The magnesium solubility was not so clearly changed with the variation of ammonium level when magnesium ammonium phosphate was used as a substrate.

It was found in all trials that phosphorus more readily dissolved than magnesium. The solubility of phosphorus was substantially higher than magnesium when magnesium phosphate was used as a substrate.

Discussion

From the results of this experiment, it is evident that a decrease of pH would increase the solubilities of magnesium phosphate and magnesium ammonium phosphate. This observation is supported by the report of Johnson[46] that a decrease of pH would result in an increase in the capacity of the liquid phase to hold magnesium, phosphate and ammonium in salt solution when equilibrated with solid magnesium ammonium phosphate.

Elliot et al.[23] also reported that the solubility of magnesium ammonium phosphate increased with increasing hydrogen ion concentration. As previously mentioned, urine pH values were decreased in pH ranges from 0.8 to 1.4 by the administration of ammonium chloride in wethers. The acidified urine, when given ammonium chloride, seemed to increase the solubility of urinary calculi. This may support the assumption by Bushman et al.[10,11] that the mode of action of ammonium chloride would be through a reduction of urine pH in sheep.

When the calcium concentration increased in buffer solutions, the phosphorus level in supernatant liquid tended to decrease after the equilibration with magnesium phosphate. This result may be caused by the formation of a complex of calcium and phosphorus in the buffer solution. When urine calcium levels are heightened by giving ammonium chloride, calcium and phosphorus may combine in urine and the decreased urine phosphorus level may interfere

with the formation of phosphatic urinary calculi. However, this protective effect of heightened urine calcium level against urolithiasis would be limited because the changing of solubility of phosphorus when the variation of calcium levels was not so severe. If the urine concentration of calcium and phosphorus are fairly high, then urinary calculi of calcium phosphate type might be formed. However, when a high concentrate ration is given to cattle and sheep, the urinary calculi of calcium phosphate type will seldom be observed because a urine calcium level is generally very low.

It was observed in this experiment that the solubility of magnesium ammonium phosphate increased slightly as the sodium concentration elevated in buffer solutions. Johnson et al.[46] reported that increasing the concentration of sodium chloride, sodium sulfate and sodium citrate had caused increases of the solubility of magnesium ammonium phosphate through the elevation of ionic strength. Since the increase of the solubility of magnesium ammonium phosphate was slight, the giving of ammonium chloride to the elevated urine sodium level would not correlate with the prevention of phosphatic urolithiasis. This indication is supported by the suggestion of Uesaka et al.[93] and Udall et al.[91] that there appeared to be no relationship between the urine sodium level and the occurrence of urolithiasis.

The solubility of magnesium ammonium phosphate appeared to increase slightly by increasing the chloride concentration in

buffer solutions. Udall et al.[91,92] postulated that the administration of sodium chloride reduced the occurrence of urolithiasis due to the increased urine chloride ion which caused ion competition around calculi. However, the positive relationship between the solubilities of magnesium phosphate and magnesium ammonium phosphate, and chloride concentrations in buffer solutions, was not definitely found in this experiment. Accordingly, this result may not support the hypothesis of Udall et al.[91,92].

It is known that the urine ammonium level is increased with metabolic acidosis in ruminants[77,78]. But the urine ammonium level will scarcely exceed 600 mg% even if the urine ammonium level would be heightened with acidosis. Therefore, the elevated ammonium level in urine may serve the forming of phosphatic urinary calculi, but will not act on the prevention or treatment of urolithiasis.

It was observed in all trials that phosphorus was more readily dissolved in buffer solutions than magnesium. This cause was not definitely known, but it was suggested that phosphorus and magnesium would not be dissolved simultaneously and magnesium phosphate and magnesium ammonium phosphate would be converted to other compounds. The conversion to other compounds may be more obvious in magnesium phosphate than in magnesium ammonium phosphate.

Among the changes of urine composition, which are induced

by the administration of ammonium chloride, the decreased urine pH will play the most important role in the prevention of urolithiasis through increasing the solubility of phosphatic urinary calculi.

Summary

The mechanism of ammonium chloride on the prevention of urolithiasis in fattening cattle was examined in vitro through the solubility of magnesium phosphate and magnesium ammonium phosphate in tris-maleate buffer solution.

Two grams of magnesium phosphate or magnesium ammonium phosphate were added to 300 ml aliquats of buffer solution in tightly stoppered flasks. These flasks were incubated at 39° C and were shaken on a mechanical shaker during 6 hours. Samples of 5 ml were taken from each flask at intervals of 0, 1, 2, 4, and 6 hours after shaking.

When pH values were decreased in buffer solutions, the solubilities of magnesium phosphate and magnesium ammonium phosphate elevated clearly. Phophorus concentrations in supernatant liquids tended to be decreased by increasing the calcium concentration in aliquots of buffer solution. This result would be due to the formation of complex of calcium and phosphorus.

Only slight increases in the solubilities of magnesium phosphate and magnesium ammonium phosphate were induced by adding of sodium salts. In the ranges from 0 mg% to 200 mg%, and from 0 to 600, the solubilities of magnesium phosphate and magnesium ammonium phosphate tended to be decreased respectively with increasing the concentration of ammonium.

It was suggested from these results that, among the effects of ammonium chloride, the decreasing of urine pH would play the most important role on the prevention of urolithiasis in fattening cattle.

GENERAL DISCUSSION AND CONCLUSION

Urinary calculi were found in the kidney, the bladder, the urethra, the prepuce and the prepuital hair of fattening cattle fed with a high concentrate ration, and the chemical composition of these calculi consisted mainly of magnesium phosphate or magnesium ammonium phosphate. Among these urinary calculi, the calculi which lodged in the urethra seems to cause dysuria, which may lead urolithiasis. Although the calculi in prepuce also cause dysuria, urination is restored to normal after the removal of calculi.

It was shown by chemical analysis and microscopic examination that there were some differences between calculi in the kidney and those in the bladder and the urethra. This result may suggest that the process of calculi formation in the kidney would be somewhat different from that in the bladder and the urethra.

It was not definitely known whether the calculi in the bladder were made of micro calculi, which moved from the kidney, or not. The calculus in the bladder, which had many layers in it, would become enlarged by the addition of minerals. Strong morbid changes of renal tubule were found in steers which had urolithiasis. In a vitamin A deficient wether, there was a considerably large amount of desquamated epithelium cells and some of sand-like micro calculi in the pelvis of kidney. It may be conceivable that desquamated epithelium cells and/or bacterial cells may become the nuclei of calculi, which grow up and become

calculi in renal tubule or renal pelvis. In addition, mucopolysaccharide seems to play a role to concrete minerals because PAS positive materials were found in calculi.

It may be possible that calculi in the bladder moved into the urethra and formed calculi in the urethra since the mineral composition and characteristics of urinary calculi in the bladder and the urethra were similar.

From the morphological findings and chemical analyses of calculi, the elevation in urine phosphorus and magnesium concentrations, nuclei of calculi, mucopolysaccharide and urine ammonia concentration may be major causes to form urinary calculi, while the decreased urine pH seems to act as a preventive factor of urolithiasis.

The inducing factors to change urine composition are as follows.

Dietary factors : The occurrence of urolithiasis was found in a latter half of the fattening period when a high concentrate ration was given. A concentrate ration commonly contains high phosphorus and low calcium in comparison with roughage. Therefore, a dietary mineral imbalance, low calcium to phosphorus ratio, will be induced when a high concentrate - low roughage ration is given. There may be a positive relationship between dietary phosphorus content and urine phosphorus concentration. And the urine phosphorus level was evidently increased as lowering dietary calcium to phosphorus ratio.

A concentrate ration, especially wheat bran and rice bran, contains a high level of magnesium. And so the urine magnesium level may be also increased in cattle given a high concentrate ration. The incidence of urinary calculi was found in steers when urine phosphorus, magnesium and sodium levels increased. And the urinary calculi from cattle in Japan were shown to be mainly made of phosphorus and magnesium. Therefore, it is conceivable that the increase of urine phosphorus and magnesium levels which originated from dietary mineral imbalances seem to correlate with the incidence of urinary calculi.

The deficiency of vitamin A may be induced by giving a high concentrate-low roughage ratio. In particular, the vitamin A deficiency is likely to occur when a low quality of roughage is fed in the winter season. As shown in this study, the renal nephrosis and the increase of urine phosphorus were found in wethers which had vitamin A deficiency. The desquamated epithelium cells induced by vitamin A deficiency may become the nuclei of urinary calculi. It was shown by Uesaka et al.[93] that cattle affected by urolithiasis have not always a vitamin A deficiency. Therefore vitamin A deficiency may become a causative factor of urinary calculi, but may not be a essential one.

Physiological factors : A mild form of acidosis which was manifested with the decrease in urine pH and the increase in urine phosphorus level was observed in wethers given a high concentrate ration. It was reported by Scott et al.[77] that the urine ammonium level was heightened in sheep which had acidosis. The relationship

between the increase of urine ammonium level and the incidence of urinary calculi was not definitely known from this study. Munakata et al.[60] reported the addition of ammonium salts to fresh urine increased the precipitation of urine sediment.

The decrease in urine pH appeared not to accelerate the formation of urinary calculi but prevent the calculi formation. This consideration may be supported by the finding in chapter 7 that the main function of ammonium chloride was to lower the urine pH. Eaton[20], in his early work with rats, indicated that the addition of an excess acid or basic sodium phosphate to the food was followed by extensive pathological changes in the kidney of rat. Renal pathological changes may become the cause of the formation of urinary calculi similar to the case of vitamin A deficiency.

Therefore the mild form of acidosis may be correlated with the formation of urinary calculi through the increase of urine phosphorus level or renal morbid changes.

It was shown that urine volume decreased when the ratio of concentrate to roughage was increased. The concentration of urine volume will cause the increase of urine phosphorus, magnesium and mucoprotein levels because there appears to be a negative association between urine volume and urine composition. It was observed in Japan[63] that the occurrence of urolithiasis in cattle increased in winter and spring. It may be possible to consider that the high incidence of urolithiasis resulted from a decreased amount of roughage intake or a lowered winter water intake.

Other factors : It was always found that urolithiasis occurred in some animals and did not occur in others when a same ration was given. Field and Sattle [28] observed in the experiment using twin cattle that urine phosphorus concentration was firmly accepted as the genetic control. Although the lodging of urinary calculi in the urethra will lead to the occurrence of urolithiasis, there may be individual differences in the size of urethra.

The diagram of causative factor of the incidence of urinary calculi is shown in figure 1. It must be primarily considered that not one factor alone is the cause of incidence of urinary calculi but many complicated factors.

As the author know the causative factors of urolithiasis, there may be many ways for its prevention and treatment. The treatment and preventive method may be the followings ;

1 : Feeding with adaquate amount of roughage, especially green forage.

2 : Adjusting the dietary calcium to phosphorus ratio by the addition of calcium carbonate to the ration.

3 : The administration or injection of vitamin A supplement.

4 : Giving adequate amounts of drinking water, the administration of salt for stimulating water intake or dieuretic agents.

5 : Early removal of urinary calculi in the prepuce and at the prepuital hair.

6 : The administration of ammonium chloride as soon as the incidence of urolithiasis is observed.

7 : Excluding the calves, on fattening practice, which have urinary calculi in the prepuital hair because such calves have, in many instances, hereditary characteristics likely to induce urolithiasis.

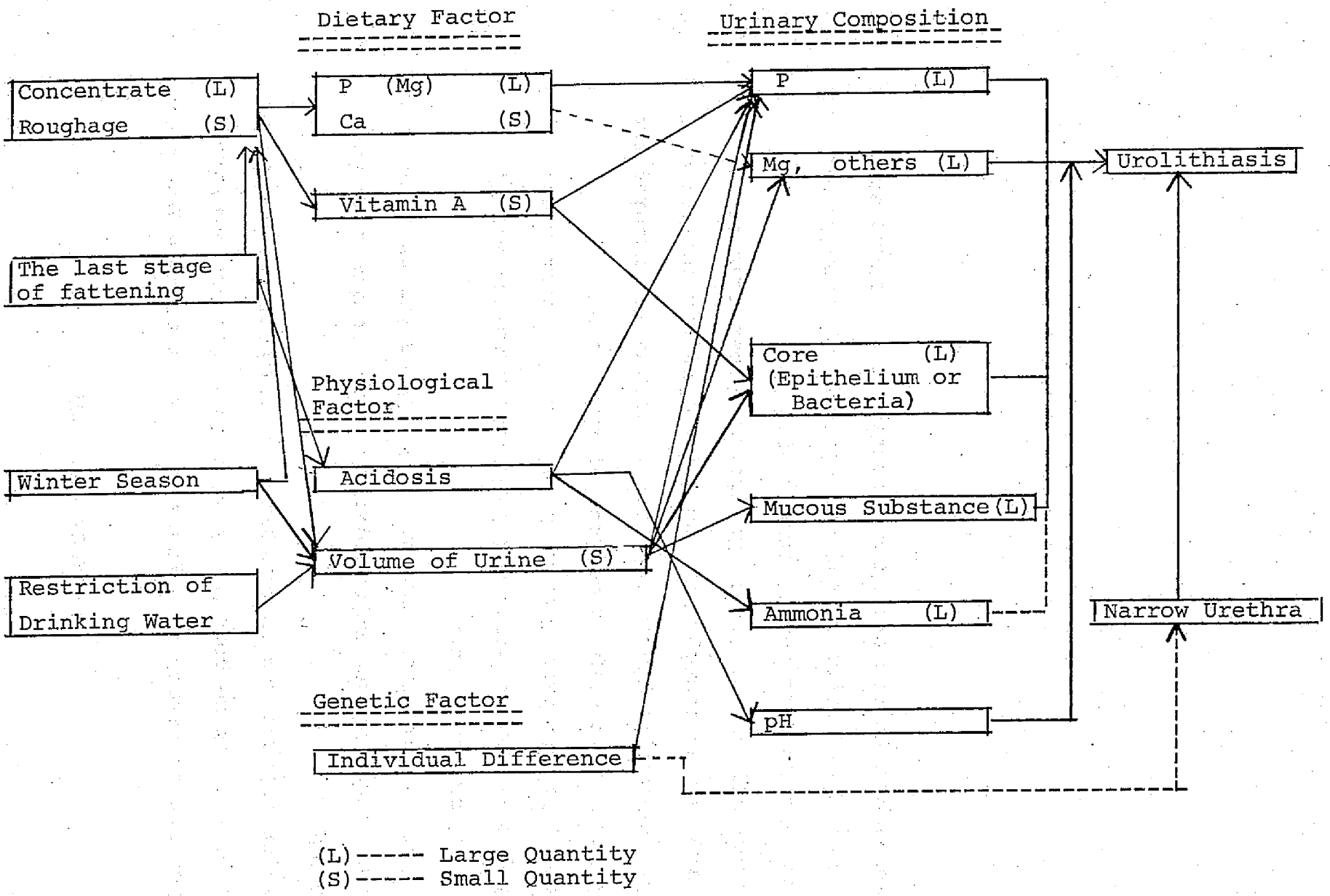


Fig. 1. Factors Influencing to Urolithiasis

ACKNOWLEDGEMENT

The author wishes to express his most sincere appreciation and respect to Professor, Dr. R. Kawashima for his valuable advice, assistance and encouragement throughout the course of this study.

The author wishes to express his deep gratitude to Dr. S. Uesaka, Dr. Y. Nishikawa and Dr. K. Namikawa for their valuable advice and encouragement.

Appreciation is expressed to Mr. G. Mishiro, Mr. T. Yoshimoto, Mr. T. Kida, Mr. H. Matui, Mr. S. Sakurai and Mr. K. Miyoshi for their help in conducting this study.

The author is grateful to Mr. T. Kamiya for reading and improving the manuscript. The help given by the colleague in the laboratory of the Animal Nutrition, Department of Animal Science is also gratefully acknowledged.

The author wishes to express his appreciation to his wife for her encouragement during the course of gratitude study.

REFERENCES

1) Anderson, A. J., and N. F. Maclagan, Biochem J 59; 638-644. 1955.

2) Aston, B. C., New Zealand Dept of Agric Ann Rep, 60-65. 1934-1935.

3) Association of Vitamin Chemists, Inc; Methods of Vitamin Assay (2nd Ed.) Interscience Publishers, Inc., New York. 1951.

4) Baker, S. B., and W. H. Summerson, J Biol Chem 138; 535-554. 1941.

5) Beeson, W. M., R. J. Pence,and G. C. Holm, Am J Vet Res 4; 120-126. 1943.

6) Ben-Ghedalia, H. Tagari, S, Zamwel, and A. Bondi, Br J Nutr 33; 87-94. 1975.

7) Blout, W. P., Vet J 87; 561-576. 1931.

8) Bushman, D. H., R. J. Emerick, and L. B. Embry, J Anim Sci 24; 671-675. 1965.

9) Bushman, D. H., R. J. Emerick, and L. B. Embry, J Nutr 87; 499-504. 1965.

10) Bushman, D. H., L. B. Embry, and R. J. Emerick, J Anim Sci 26; 1199-1204. 1967.

11) Bushman, D. H., L. B. Embry, and R. J. Emerick, J Anim Sci 27; 490-496. 1968.

12) Chandler, P. T., and R. G. Cragle, Proc Soc Exp Biol Med 111; 431-436. 1962.

13) Cornelius, C. E., J. E. Moulton, and B. Macgown, Am J Vet Res 20; 863-871. 1959.

14) Cornelius, C. E., and J. J. Kaneko, Clinical Biochemistry of Domestic Animals; 348. Academic Press. New York and London. 1963.

15) Crookshank, H. R., F. E. Keating, J. H. Jones, and R. E. Davis, J Anim Sci 19; 595-600. 1960.

16) Crookshank, H. R., J. D. Robbins, and H. O. Kunkel, J Anim Sci 26; 1179-1185. 1967.

17) Dang, H. C., M. S. thesis, Montana State College, Bozeman. 1959.

18) Dirksen, G., Physiology of Digestion and Metabolism in the Ruminant; 612-629. Butterworths. London. 1969.

19) Dunlop, H., and B. Hammond, Annals N Y Acad Sci 118; 1109-1131. 1965.

20) Eaton, M. M., and J. Oliver, J Exp Med 61; 319-333. 1935.

21) Elam, C. J., B. H. Schneider, and W. E. Ham, J Anim Sci 15; 800-810. 1956.

22) Elam, C. J., B. H. Schneider, and W. E. Ham, Proc Soc Expl Biol Med 95; 769. 1957.

23) Elliot, J., W. O. Robert, F. Shopp, and L. Lewis, J Urol 80; 269-271. 1958.

24) Emerick, R. J., L. B. Embry, and O. E. Oslon, J Anim Sci 19; 1025-1030. 1959.

25) Emerick, R. J., and L. B. Embry, J Anim Sci 23; 1079-1083. 1964.

26) Emerick, R. J., and L. B. Embry, J Anim Sci 22; 510-512. 1963.

27) Emerick, R. J., H. R. King, and L. B. Embry, J Anim Sci 35; 901-907. 1972.

28) Field, A. C., and N. F. Suttle, Proc Nutr Socie 29; 34 A 1970.

29) Fiske, C., and Y. Subbarow, J Biol Chem 66; 375-400. 1925.

30) Frank, F. W., R. R. Baron, L. H. Scrivner, and T. B. Keith, Am J Vet Res 22; 899-901. 1961.

31) Gill, W. B., B. Finlayson, and C. W. Vermeulen, J Urol 82 ; 256-260. 1964.

32) Goodall, E. D., and R. N. B. Kay, J Physiol 176; 12-23. 1965.

33) Hawkins, W. W., J Am Vet Med Assn 147; 1321-1323. 1965

34) Handler, P., D. V. Cohn, and W. J. D. Emaria, Am J Physiol 165; 434-441. 1951.

35) Hiatt, H. H., and D. D. Thompson, J Clin Invest 36; 557-565. 1957.

36) Hiatt, H. H., and D. D. Thompson, J Clin Invest 36; 573-580. 1957.

37) Higgins, C. C., J Am Vet Med Assn 118; 81-85. 1951.

38) Hoar, D. W., R. J. Emerick, and L. B. Embry, J Anim Sci 29 ; 647-652. 1969.

39) Hoar, D. W., R. J. Emerick, and L. B. Embry, J Anim Sci 30; 597-600. 1970.

40) Hoar, D. W., R. J. Emerick, and L. B. Embry, J Anim Sci 31; 118-125. 1970.

41) Howard, J. E., T. R. Hopkins, and T. B. Connor, J Clin Endocrinol Metabolism 13; 1-19. 1953.

42) Huber, T. L., J Anim Sci 28; 98-102. 1969.

43) Ishizuka, O., Experimental Methods of Microdiffusion Analysis, 70-75. Nankodo Co. Ltd., Tokyo Japan. 1968.

44) Jigyo Gaiyo, Shokuniku Eisei Kensasho in Kyoto City. 1974.

45) Johnson, D. W., L. S. Palmer, and J. W. Nelson, Vet Med 34; 353. 1940.

46) Johnson, R. G., J Urol 81; 681-690. 1959.

47) Kachiku Kyosai Tokei, Ministry of Agriculture and Forestry. Tokyo Japan. 1974.

48) Kaneko, I. E., J. W. Tobiska, and H. B. Asland, Colorado Agric Exper Sta Tech Bull 311; 1-42. 1943.

49) Kohlmeir, R. H., and W. Burroughs, J Anim Sci 30; 1012-1018. 1970.

50) Krishnaro, G. V. G., and H. H. Draper, J Nutr 102; 1143-1146. 1972.

51) Kunkel, H. O., E. E. Whiter, L. B. Packett, and H. R. Crookshank, J Anim Sci 26; 1179-1185. 1967.

52) Law, J., U. S. Department of Agriculture, Bureau of Anim Industry, Goverment of Printing Office, Washington. 1909.

53) Lemann, J., J. R. Litzow, and E. J. Lennon, J Clin Invest 46; 1318-1328. 1967.

54) Lennon, E. J., and W. F. Piering, J Clin Invest 49; 1458-1465. 1970.

55) Leuker, C. E., and G. P. Lofgreen, J Nutr 74; 233-238. 1961.

56) Levine, R., and R. Luft, Advances in Metabolic Disorders Vol. 1; 52-54. Academic Press. New York and London. 1964.

57) Lindley, C. E., E. D. Taysom, W. E. Ham, and B. H. Schneider, J Anim Sci 12; 704-714. 1953.

58) Martin, E. H., and R. Jones, Am Heart J 62; 206-210. 1961.

59) Mathams, R. H., and A. K. Sutherland, Australian Vet J 27; 68-69. 1951.

60) Munakata, K., K. Ikeda, and H. Suda, Nat Inst Anim Hlth Quart 14; 89-96. 1974.

61) Newsom, I. E., J Am Vet Med Assn 92; 495-502. 1938

62) Newsom, I. E., J. W. Tobiska, and H. B. Asland, Colorado Agric Exper Sta Tech Bull 311; 1-42. 1943.

63) Oshikawa, M., Kachiku Kyosai 106; 13-16. 1972.

64) Packett, L. V., and J. P. Hauschild, J Nutr 84; 195-190. 1964.

65) Packett, L. V., and S. P. Coburn, Am J Vet Res 26; 112-119. 1965.

66) Perry, S. C., R. G. Cragle, and J. K. Miller, J Nutr 93; 283-290. 1967.

67) Pfeffer, E., A. Thompson, and D. G. Armstrong, Br J Nutr 24; 197-204. 1970.

68) Pitts, R. F., Fed Proc 7; 418-426. 1948.

69) Pontius, B. E., R. H. Carr, and L. P. Doyle, J Agric Res 42; 433-446. 1931.

70) Prien, E. L., J Urol 73; 627-635. 1955.

71) Reed, W. D. C., R. C. Elliot, and J. P. Topps, Nature 208; 953-954. 1965.

72) Ricketts, R. E., D. E. Weinman, J. R. Campbell, and M. E. Tumbleson, Am J Vet Res 31; 1023-1026. 1970.

73) Robbin, J. D., H. O. Kunkel, and H. R. Crookshank, J Anim Sci 24; 76-82. 1965.

74) Rumsey, T. S., P. A. Pitman, J. Bond, and R. R. Oltzen, J Anim Sci 31; 608-616. 1970.

75) Schmidt, H., Am J Vet Res 2; 373-389. 1941.

76) Schneider, B. H., E. D. Taysom, and W. E. Ham, Wash Agr Exp Sta Cir, 203. 1952.

77) Scott, D., F. G. Whitelaw, and M. Kay, Q Jl Exp Physiol 56; 16-32. 1971.

78) Scott, D., Q Jl Exp Physiol 56; 33-40. 1971.

79) Smith, A. H., M. Kleiber, A. L. Black, and G. P. Lofgreen, J Nutr 58; 95-111. 1955.

80) Smith, R. H., Biochem J 83; 151-163. 1962.

81) Smith, R. H., J Biochem 83; 151-160. 1969.

82) Somogyi, M., J Biol Chem 160; 61-73. 1945.

83) Storry, J. E., J Agric Sci 57; 97-102. 1961.

84) Suby, H. I., J Urol 68; 96-100. 1962.

85) Swingle, K. F., and H. Marsh, Am J Vet Res 14; 16-18. 1953.

86) Swingle, K. F., and H. Marsh, Am J Vet Res 17; 415-424. 1956.

87) Telle, P. P., and R. L. Preston, J Anim Sci 33; 698-705. 1971.

88) Topps, J. H., W. D. C. Reed, and R. C. Elliot, J Agric Sci 66; 233-239. 1966.

89) Udall, R. H., Am J Vet Res 20; 426-429. 1959.

90) Udall, R. H., Am J Vet Res 23; 1241-1245. 1962.

91) Udall, R. H., and F. H. C. Chow, Cornell Vet 53; 538-544. 1965.

92) Udall, R. H., C. L. Seger, and F. H. C. Chow, Cornell Vet 55; 198-203. 1965.

93) Uesaka, S., R. Kawashima, K. Namikawa, and G. Mishiro, Research Bulletin of Takahara Experimental Station in Kyoto University, No.19. 1967.

94) Uesaka, S., R. Kawashima, J. Saida, H. Yano, T. Yokota, and S. Yoshii, Research Bulletin of Laboratory of Animal Breeding and Husbandry in Kyoto University, No. 300. 1971.

95) Uhart, B. A., and F. D. Carrol, J Anim Sci 26; 1195-1198. 1967.

96) Vipperman, P. E., R. L. Preston, L. D. Kinter, and W. H. Phander, J Nutr 97; 449-462. 1968.

97) Volhard, C., and S. Harvey, Archives of Internal Medicine, 6; 12. 1910.

98) Webb, K. E., G. E. Mitchell, C. O. Little, and G. H. Schmidt, J Anim Sci 27; 1657-1662. 1968.

99) Webb, K. E., G. E. Mitchell, and C. O. Little, J Anim Sci 30; 941-943. 1970.

100) Wise, M. B., R. W. Harvey, B. R. Haskins, and E. R. Barrick, J Anim Sci 27; 1449-1461. 1968.

101) Woelfel, G. G., R. C. Hall, M. C. Cahlhoun, J. E. Rousseau, and H. D. Eaton, J Dairy Sci 48; 1364-1352. 1965

102) Woodruff, A. D., Vet Med 29; 214-215. 1934.

103) Yamano, K., Saishin Kachiku Naikagaku, 212-214. Nankodo Co. Ltd., Tokyo Japan. 1970.

104) Yang, M. G., and J. W. Thomas, J Nutr 87; 444-458. 1965.